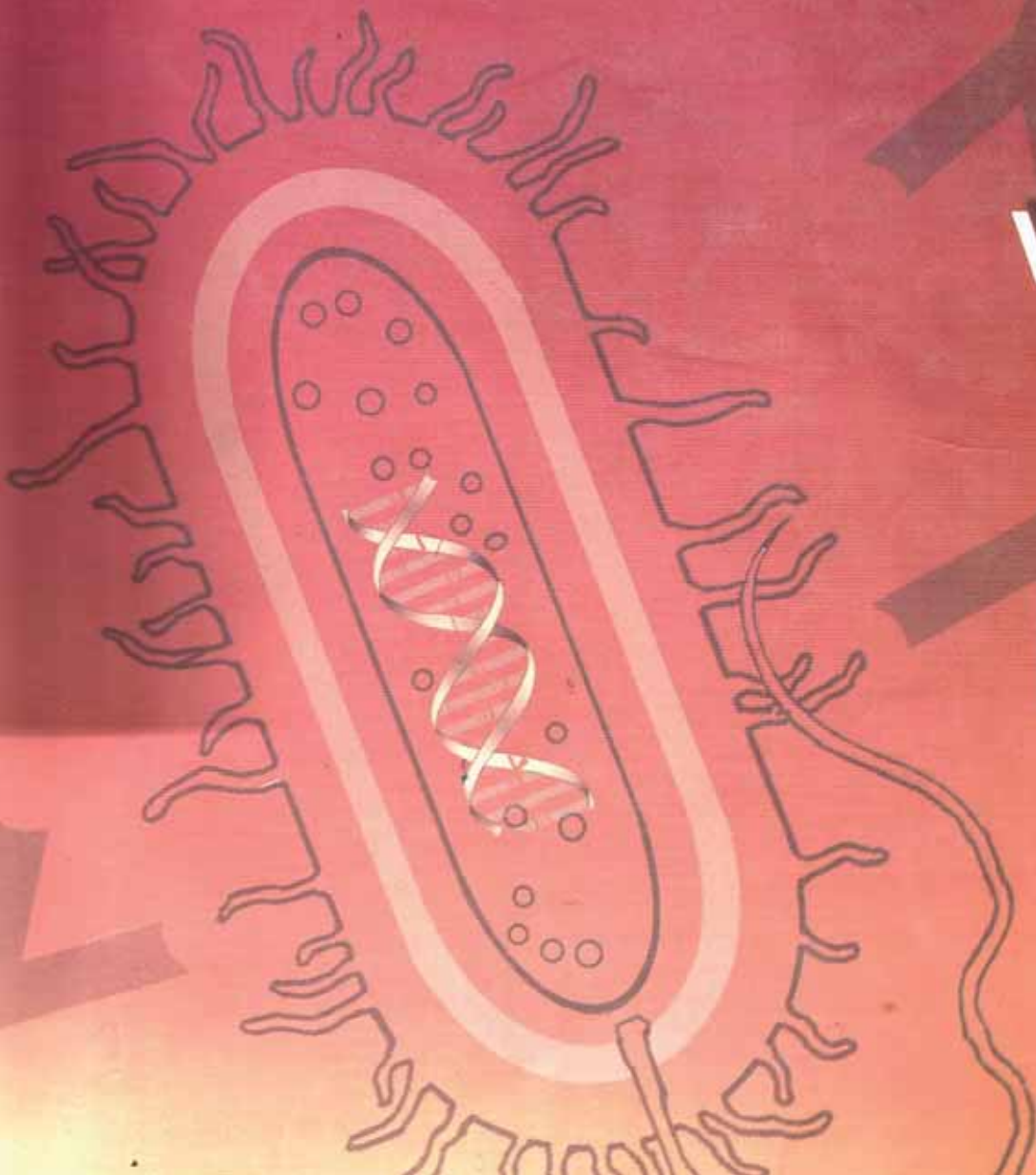


Essential Medical Microbiology and Immunology

Vol I



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P R E F A C E

This book is intended to be a comprehensive and up to date guide to medical microbiology and immunology in the most reliable, attractive, illustrated manner for undergraduate medical students, as well as a guide for postgraduates preparing for higher degrees.

It is designed to cover the four aspects of medical microbiology and immunology (Bacteriology, Mycology, Virology, and Immunology) in three separate volumes, each navigates the reader to this ever expanding science.

- *Vol. I : General Microbiology and Immunology.*
- *Vol. II : Systematic Bacteriology and Mycology.*
- *Vol. III : Systematic Virology, Applied Microbiology and a Guide to Practical Microbiology.*

In preparing this text, the primary objective of our panel was to supply the reader with a concise updated source reflecting the tremendous progress in our knowledge in the fascinating field of microbiology and immunology; it is our sincere hope that it can fulfil this goal.

The Authors

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Chapter 1

INTRODUCTION TO MICROORGANISMS

Microorganisms are generally unicellular i.e. the whole organism is one cell. In such cases, a single microbial cell performs all the functions required to maintain itself and propagate.

Microorganisms may be classified in the following 8 large biological groups:

1. Algae.
2. Protozoa.
3. Slime moulds.
4. Fungi.
5. Bacteria.
6. Archaeobacteria.
7. Viruses.
8. Prions.

neither eukaryotic nor prokaryotic bec. it's not a cell
obligate intracellular parasite

The algae (excluding the blue-green algae), the protozoa, slime moulds and fungi include the larger and more highly developed microorganisms; their cells have the same general type of structure and organization, described as eukaryotes, as that found in higher plants and animals. The bacteria, including organisms of the mycoplasma, rickettsia and chlamydia groups, together with the related blue-green algae, comprise the smaller microorganisms with a simpler form of cellular organization described as prokaryotes.

The most outstanding character of eukaryotic cells (eu=true; karyote=nucleus) is a distinct nucleus, surrounded by a membrane that separates it from the other contents of the cell. Prokaryotic cells (pro=before), on the other hand, do not contain a membrane-bound nucleus. Instead, their hereditary material is suspended in a portion of cytoplasm called nucleoid or nuclear region. They are also devoid of mitochondria and other membrane bound organelles.

Defines:-
The viruses are one of the smallest infective agents; they have no cell structure. Viruses are obligate intracellular parasites; they require the biological machinery of a host cell for reproduction and survival. Even simpler are viroids, protein-free fragments of single-stranded circular RNA that cause disease in plants.

▶▶ Prions are described as infectious proteins devoid of nucleic acid. → mad cow disease

Since the algae, slime moulds and archaeobacteria are not thought to contain species of medical or veterinary importance, they will not be considered further. Blue-green algae do not cause infection, but certain species produce potent toxins that may affect people or animals drinking polluted water.

Perenae → no harm → no disease non infectious

- ① Stain
- ② size
- ③ shape
- ④ arrangement
- ⑤ Motility

Morphology

chapter 2

BACTERIA: THEIR STRUCTURE AND ORGANIZATION

Bacteria were first discovered by **Leeuwenhoek 1674**. They are among the most widely distributed forms of life. They are found in air, water and soil. They are also found in or on the human body, animals and plants.

- ⑤ Motility
- ⑥ Arrangement

- ① Stain
- ② size
- ③ shape
- ④ Content

Bacterial Morphology

5 shape

Bacteria are differentiated into major categories, based on their morphological features such as shape, size, arrangement and staining characteristics.

Bacterial Size

Most bacteria range in size from $0.2-1.2 \mu\text{m}$ in width and $0.4-14 \mu\text{m}$ in length.

Bacterial Shape and Arrangement

(A) **Cocci** (singular: **coccus**): are spherical organisms with a wide variety of arrangements, e.g.:

1. Diplococci: pairs of cells, e.g. *Neisseria*.
2. Irregular grape-like clusters, e.g. *staphylococci*.
3. Chains of four or more, e.g. *streptococci*.

(B) **Bacilli** (singular: **bacillus**=stick): are rod-shaped organisms. These cells may occur singly, in pairs or in chains. Some bacilli are short (*coccobacilli*), others are curved (*vibrios*).

(C) **Spiral bacteria**: are divided into two groups, **spirilla** which are rigid and **spirochaetes** that are flexible.

The arrangement of cells is determined by the planes of division. For example, the cocci that divide along a single plane produce diplococci or chains, e.g. streptococci, while those that divide on many planes produce clusters, e.g. staphylococci.

Staining Characteristics

There are two kinds of stains: simple and differential. **Simple stains** employ a single dye like methylene blue, crystal violet or fuchsin. Cells and structures stained with them give the same colour. Therefore, they only reveal the characteristics of size, shape and arrangement. **Differential stains** require more than one dye, and distinguish between different types of bacteria by giving them different colours.

Gram's stain is the most important differential stain in clinical microbiology. It divides bacteria into **Gram-positive** (violet-staining) and **Gram-negative** (red staining). The second important stain is Ziehl-Neelsen stain. It is used to identify the acid-fast bacilli of the genus *Mycobacterium*.

chain
grape

Bacterial Ultra-Structures and their Functions

All bacteria have a **nucleoid**, ribosomes and a cytoplasmic membrane. Most bacteria also have a cell wall and some are further enveloped by a capsule or slime layer. Some types of bacteria have also cytoplasmic inclusions and various appendages as flagella and pili. The final details of subcellular structures are best revealed by electron microscopy (Fig. 1).

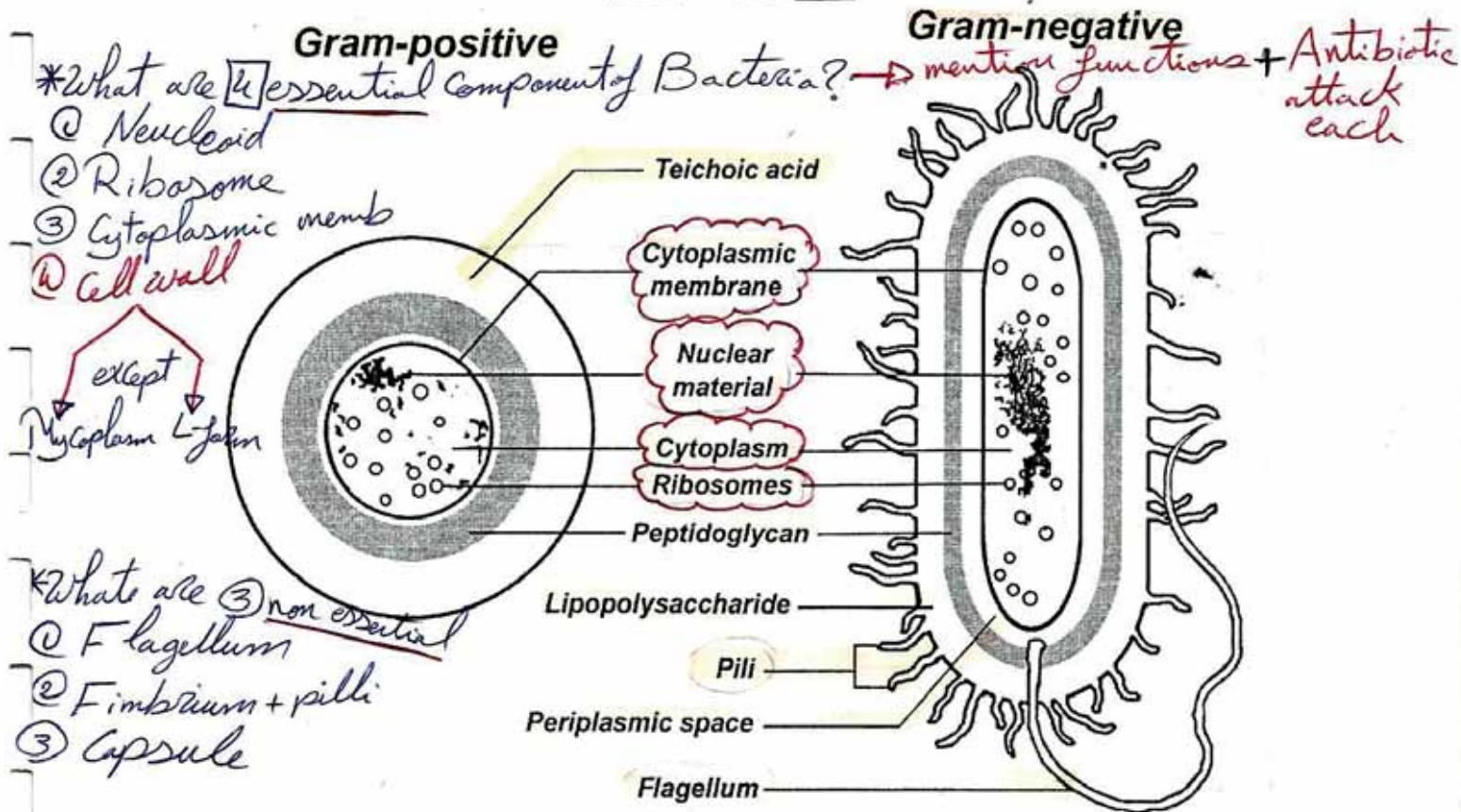


Fig. (1): Schematic representation of a Gram-positive and a Gram-negative bacterium.

Cytoplasm

Few morphologically distinct

components can be found within the cytoplasm:

Prokaryotic

Nucleoid: Genetic information of a bacterial cell is contained in a single circular molecule of double-stranded DNA, which constitutes the bacterial chromosome. It is 1 mm long and is packed into a supercoiled state inside the cell. → bacteria 0.2 μm

Plasmids: In many bacteria, additional genetic information is contained on plasmids which are small circular extrachromosomal DNA molecules that can replicate independently of the chromosome.

Ribosomes: They are the site of protein synthesis in the cell. Ribosomes consist of protein and RNA. Prokaryotic ribosomes have a sedimentation constant of 70S, smaller than the 80S ribosomes of eukaryotes. This difference makes bacterial ribosomes a selective target for antibiotic action.

Handwritten notes:

- G.R. Ribosome selective target for antibiotic
- ① sedimentation
- ② difference

Inclusion granules: These are granules of nutrient materials, usually phosphates, sulphur, carbohydrates and lipids. Energy reserves are usually stored as glycogen, starch or poly- β -hydroxybutyrate. Phosphate is stored in metachromatic or volutin granules, which are used for synthesis of ATP.

Mesosomes: These are complex invagination of the cytoplasmic membrane. They are involved in cell division and sporulation. They also have a function analogous to the mitochondria in eukaryotes providing a membranous support for respiratory enzymes.

Cytoplasmic Membrane

In bacteria, as in other cells, the protoplast is limited externally by a thin elastic cytoplasmic membrane. It is a phospholipid protein bilayer similar to that of eukaryotic cells except that, in bacteria, it lacks sterols. It has the following functions

1. **Selective transport:** In bacteria, molecules move across the cytoplasmic membrane by simple diffusion, facilitated diffusion and active transport.

2. **Excretion of extracellular enzymes:**

- Hydrolytic enzymes: which digest large food molecules into subunits small enough to penetrate the cytoplasmic membrane.
- Enzymes used to destroy harmful chemicals, such as antibiotics, e.g. penicillin-degrading enzymes.

3. **Respiration:** The respiratory enzymes are located in the cytoplasmic membrane, which is thus a functional analogue of the mitochondria in eukaryotes.

4. **Cell wall biosynthesis:** The cytoplasmic membrane is the site of:

- The enzymes of cell wall biosynthesis.
- The carrier lipids on which the subunits of the cell wall are assembled.

5. **Reproduction:** A specific protein in the membrane attaches to the DNA and separates the duplicated chromosomes from each other. In addition, a septum is formed by the cytoplasmic membrane to separate the cytoplasm of the two daughter cells.

6. **Chemotactic system:** Attractants and repellants bind to specific receptors in the cytoplasmic membrane and send signals to the cell's interior. The cell then responds to the surface message.

Cell Wall

Surround cytoplasmic memb. 10-25 nm thick & Rigid

The bacterial cell wall is the structure that immediately surrounds the cytoplasmic membrane. It is 10-25 nm thick, strong and relatively rigid, though having some elasticity.

Structure of the cell wall

The cell wall of bacteria is a complex structure. Its impressive strength is primarily due to peptidoglycan (synonym: murein or mucopeptide). Peptidoglycan is a complex polymer consisting of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) that is unique to bacteria. A set of identical tetrapeptide side chains are attached to NAM.

Besides peptidoglycan, additional components in the cell wall divide bacteria into Gram-positive and Gram-negative (Fig. 1).

Gram -ve & Gram +ve ← Cell wall

Gram-positive cell wall is composed of:

Peptidoglycan: There are as many as 40 sheets of peptidoglycan, comprising up to 50% of the cell wall material. Despite the thickness of peptidoglycan, chemicals can readily pass through.

Teichoic acids: They are the polymer of ribitol or glycerol phosphate. They are found in the cell wall of most Gram-positive bacteria. Teichoic acids and cell wall associated proteins are the major surface antigens of the Gram-positive bacteria.

Gram-negative cell wall is composed of:

Peptidoglycan: It is much thinner, composed of only one or two sheets comprising 5-10% of the cell wall material.

Outer membrane: It is phospholipid protein bilayer present external to the peptidoglycan layer. The outer surface of the lipid bilayer is composed of molecules of lipopolysaccharide (LPS) which consists of a complex lipid called lipid A chemically linked to polysaccharides. Lipid A of the LPS forms the endotoxin of the Gram-negative bacteria, while polysaccharides are the outermost molecules of the cell wall and are major surface antigens of the Gram-negative bacterial cell (somatic or O antigen).

Periplasmic space: It is the space between the cytoplasmic and outer membranes. It contains the peptidoglycan layer and a gel-like solution of proteins.

Functions of the cell wall

1. It maintains the characteristic shape of the bacterium.
2. It supports the weak cytoplasmic membrane against the high internal osmotic pressure of the protoplasm (5-25 atm.).
3. It plays an important role in cell division.
4. It is responsible for the staining affinity of the organism.

Wall deficient variants

a- Mycoplasma: It is the only group of bacteria that exists naturally without cell wall. Mycoplasmas do not assume a defined recognizable shape, because they lack a rigid cell wall. These organisms are naturally resistant to cell wall inhibitors, such as penicillins and cephalosporins.

b- L. Forms: They are wall defective or wall deficient bacteria.

- "L" stands for Lister Institute in London, where they were first discovered.
- "L" forms may develop from cells that normally possess cell wall, when they are exposed to hydrolysis by lysozyme or by blocking peptidoglycan biosynthesis with antibiotics, such as penicillin, provided that they are present in an isotonic medium.
- Some L. forms resynthesize their walls once the inducing stimulus is removed. Others, however, permanently lose the capacity to produce a cell wall.
- L. forms may survive antibiotic therapy. Their reversion to the walled state can produce relapses of the overt infection.

→ endotoxin from Lipid A of Lipopolysaccharide

what is endotoxin?

What is the cause and effect of cell wall rigidity?

① Peptidoglycan

never resynthesize cell wall

is into air

(Isotonic only)

Induced

ایک ایسی حالت جس میں خلیہ کی دیوار نہیں بنتی اور وہ لکڑی کی طرح ہوتی ہے۔

→ Mostly poly
saccharide

±

Capsule and Related Structures *outside cell wall but inside host in vivo*

Many bacteria synthesize large amount of extracellular polymer that collects outside the cell wall to form an additional surface layer. This layer is formed only inside the host (in-vivo). With one known exception (the polypeptide capsule of *Bacillus anthracis*), the extracellular material is made of polysaccharides.

Capsule: It is such a layer that adheres to the surface of the cell and forms a well-defined halo when differentially stained, to be resolved with the light microscope.

Slime layer: It is a surface layer that is loosely distributed around the cell.

Glycocalyx: It is a loose meshwork of polysaccharide fibrils extending outwards from the cell.

Functions: *GR. Why only in vivo = Functions*

1. It protects the cell wall against various kinds of antibacterial agents, e.g. bacteriophages, colicins, complement and lysozymes.
2. It protects the bacterial cell from phagocytosis. Hence, the capsule is considered an important virulence factor.
3. Some bacteria attach to the target surface by using their capsules or glycocalyx in order to establish infection. For instance, *Streptococcus mutans* form glycocalyx, with which the bacteria stick to the tooth enamel.

*Function of Glycocalyx?

Appendages

Several structures project through the cell wall of bacteria to form surface appendages. The most commonly observed are flagella and pili.

A- Flagella

→ ptn → Antigenic

Many genera of bacteria move by means of flagella.

- Flagella are only 20 nm in diameter, too small to be detected by light microscope. They can be demonstrated clearly with the electron microscope.
- The location and number of flagella on a cell vary according to bacterial species. Organisms may be **monotrichous** (single polar flagellum), **lophotrichous** (multiple polar flagella) or **peritrichous** (flagella distributed over the entire cell surface) (Fig. 2).
- Flagella consist of a single type of protein called flagellin which differs in different bacterial species. The flagellins are highly antigenic (H antigen).

Motile bacteria tend to migrate towards regions where there is a higher concentration of nutrients and solutes (a process known as chemotaxis) and away from disinfecting substances (negative chemotaxis).

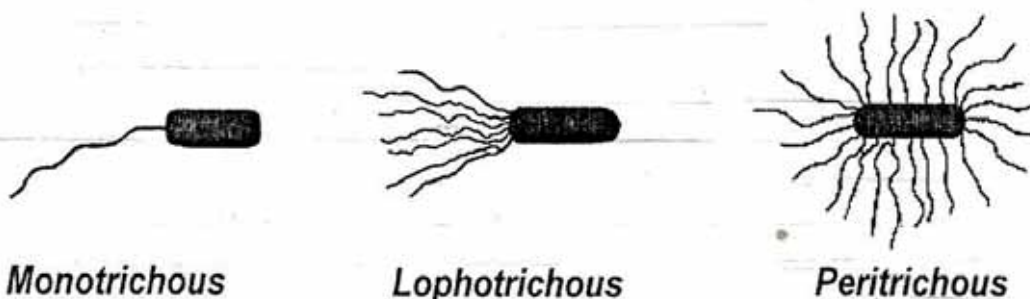


Fig. (2): Different distribution of flagella.

Axial filaments $\Rightarrow$ endoflagella

These structures are composed of two groups of fibres that originate within the opposite ends of the cell and overlap in the middle. Structurally and chemically, the fibres of the axial filaments are similar to flagella and they are sometimes called "endoflagella". Spirochaetes move by means of these axial filaments. When the cell moves, it rotates around its longitudinal axis and flexes and bends along its length.

B- Pili or fimbriae

Pili (singular: pilus) are protein tubes that extend from the cells. They are shorter and thinner than flagella and can be observed only by the electron microscope. They are composed of structural protein subunits termed pilins.

Functions:

- Adherence:** It is the function of the short pili (fimbriae) that occur in great numbers around the cell. They enable bacteria to attach to the host surfaces, thus contributing to the establishment of infection i.e. virulence factor. For instance, *Neisseria gonorrhoeae* withstands the flushing action of urine by adhering to the urethral mucosa.
- Conjugation:** A special long pilus called the sex pilus (F or fertile pilus) is involved in the transfer of DNA between bacteria, a process known as conjugation (see Chapter 6).

Bacterial Spores (Endospores)

Some bacteria, notably those of the genera *Bacillus* and *Clostridium*, develop a highly resistant resting phase or endospore that does not grow or reproduce, and exhibits absolute dormancy. A single vegetative bacterium forms a single spore by a process called **sporulation**. A single vegetative bacterium emerges from a spore during **germination**.

not replication

Sporulation $\rightarrow$ protection mechanism

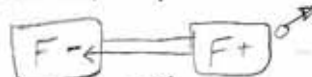
Sporulation is triggered by the onset of unfavourable environmental conditions e.g. depletion of nutrients, accumulation of metabolites or changes in the growth requirements (e.g. moisture, temperature, pH, or oxygen tension).

The cytoplasmic membrane invaginates enclosing a section of the cytoplasm that contains the bacterial chromosome, some ribosomes and other cytoplasmic materials that will be needed for germination. It acquires a thick cortex and a thin but tough outer spore coat.

Viability and resistance

Spores are much more resistant to disinfectants, drying and heating. Moist heat at 121°C for 10-20 minutes is needed to kill spores while 60°C suffices to kill vegetative forms. The marked resistance of the spores has been attributed to several factors:

- Thermal resistance is provided by their high content of Ca^{2+} and dipicolinic acid (a compound unique to endospores).
- The impermeability of their cortex and outer coat.
- Their low content of water.
- Their very low metabolic and enzymatic activity.





Germination

Endospores can respond quickly to changes in the environment returning to the vegetative state within 15 min. In the process of germination, the spores absorb water and swell, the protective coat disintegrates and a single vegetative cell emerges.

@ stain @ site @ shape @ size
SSSS S

Morphology

1. Staining: Using Gram's stain, the spore remains uncoloured and can be seen as a clear area within the stained cell. The spores can be stained using special procedures.

2. The position (Fig. 3): In relation to the body of the bacillus, the spore may be central, terminal or subterminal.

3. The shape: The spores may be oval or rounded.

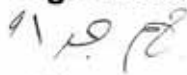
The position and shape of spores are characteristic of the species and may help in the microscopic identification of the bacterium.



Central



Terminal
e.g. *Cl. tetani*



Subterminal

oval
subterminal

Fig. (3): The position of spores.

@ size

- ① small → bulging don't occur
② Large → bulging

*function: ?

Capsule Both are essential Spore

*function: ?

- * outside cell wall
- * outside organism
- * in vivo inside host
- * polysaccharide mainly
- * polypeptide rarely
- Important for replication

- * in vitro
- * inside cell wall cytoplasm
- * high content Ca^{++} + dipicolinic acid

→ Can't replicate

Chapter 3 Growth Colonies can be clear fluid → turbid

BACTERIAL GROWTH AND PHYSIOLOGY

Growth involves an increase in the size and number of organisms. In the laboratory, bacterial growth can be seen in one of two main forms:

- 1 Development of colonies, which are the macroscopic products of 20-30 cell divisions of a single bacterium on solid media. Define Colony
- 2 Transformation of a clear fluid medium to a turbid suspension. not micro

Bacterial Reproduction = replication

Bacterial multiplication takes place by **simple binary fission**.

1. The cell grows in size usually elongates.
 2. The bacterial chromosome acts as a template for the replication of another copy.
 3. Each copy becomes attached to a mesosome on the cytoplasmic membrane.
 4. The protoplasm becomes divided into two equal parts by the growth of a transverse septum from the cytoplasmic membrane and cell wall. 3 Types of separation
- ▶ In some species, this septum splits the parent cell completely into two separate daughter cells. In others, the cell walls of the daughter cells remain continuous for sometime after division giving the characteristic arrangement, e.g. pairs, clusters or chains.

Generation time (doubling time) is the time between two successive divisions. It may be as short as 13 min. in *Vibrio cholerae* and may reach 24 h. = 1 day in *Mycobacterium tuberculosis*.

Growth Requirements

In order to grow and divide, bacteria need the following growth requirements:

1. **Nutrients**: According to the means by which a particular organism obtains energy and raw material to sustain its growth, bacteria are classified into:

a- **Autotrophs**: They can utilize simple inorganic materials, e.g. CO_2 as a source of carbon and ammonium salts as a source of nitrogen. They can synthesize complex organic substances from the simple inorganic materials. The energy required for their metabolism is predominantly derived from light or simple chemical reactions. Autotrophs are of no or little medical importance. (not pathogenic)

b- **Heterotrophs**: These bacteria, on the other hand, require organic sources for carbon, as they can not synthesize complex organic substances from simple inorganic sources. Most bacteria of medical importance are heterotrophic.

2. **Oxygen (O_2)**: According to O_2 requirements, bacteria are classified into:

a- **Strict or obligate aerobes** require oxygen for growth, e.g. *Pseudomonas aeruginosa*.

b- **Strict or obligate anaerobes** require complete absence of oxygen, e.g. *Bacteroides fragilis*. G.R.

c- **Facultative anaerobes** generally grow better in presence of oxygen but still are able to grow in its absence, e.g. staphylococci, *E. coli*, ...etc.

Facultative anaerobes → 2005 → Define + e.g.?

d- **Micro-aerophilic** organisms require reduced oxygen level, e.g. *Campylobacter* and *Helicobacter*.

e- **Aerotolerant anaerobes** have an anaerobic pattern of metabolism but can tolerate the presence of oxygen because they possess superoxide dismutase e.g. *Clostridium perfringens*.

Respiration and energy production The cellular respiration is another name of glucose catabolism. When it takes place in presence of oxygen, it is called aerobic cellular respiration. When it takes place in absence of oxygen, it is called anaerobic cellular respiration. *to produce energy*

Aerobic cellular respiration The glucose catabolism under **aerobic** conditions results in the production of energy in the form of **38 ATP** molecules. The final electron acceptor is molecular O_2 . During this type of respiration, superoxide (O_2^-) and hydrogen peroxide (H_2O_2) are formed. These molecules are highly toxic. To cope with this, aerobic organisms have developed two enzymes, superoxide dismutase and catalase, which detoxify these molecules.

Anaerobic cellular respiration: It occurs in the absence of oxygen. The final electron acceptor is an inorganic molecule such as nitrate (NO_3^-), sulfate (SO_4^{2-}), or CO_2 . The net yield of ATP molecules is less than it is with aerobic cellular respiration because nitrate, sulfate, and CO_2 are not as good at accepting electrons as oxygen. Compared to aerobes, obligate anaerobes lack superoxide dismutase and catalase and so they can not grow in presence of O_2 .

Fermentation: It is an anaerobic process, because it takes place in the absence of oxygen. It is used by facultative anaerobes when they exist in an environment that does not contain a suitable inorganic final electron acceptor (NO_3^- , SO_4^{2-} or CO_2). This is the least efficient method of generating energy.

3. **Carbon dioxide (CO_2):** The minute amount of CO_2 present in air is sufficient for most bacteria. However, certain species require higher concentrations (5-10%) of CO_2 for growth (**capnophilic**) e.g. *Neisseria spp.* and *Brucella abortus*.

4. **Temperature:**

a. **Mesophiles** are organisms able to grow within a temperature range of 20-40°C. Pathogens which replicate on or in human body are able to grow within this range, with an optimum temperature of 37°C which is the normal body temperature.

b. **Psychrophiles** (cold-loving) are capable of growth at refrigeration temperature (0-8°C), e.g. *Flavobacterium spp.* *no medical imp. bec.*

c. **Thermophiles** (heat-loving) grow best at high temperature (>60°C) e.g. *Bacillus stearothermophilus*. *no medical imp. bec.* *not our Temp.*

5. **Hydrogen ion concentration (pH):** Most microorganisms of clinical significance grow best in media whose pH is close to that of human body (pH 7.2). However, some microorganisms grow better at an alkaline pH (8-9), such as *V. cholerae*. Others, such as *Lactobacilli*, prefer media of acidic pH (4 or less).

Krebs needs hydrogen acceptor

Lactic acid normally in vagina → acidic

Growth Phases (Bacterial Growth Curve)

If a small number of an organism is placed in a suitable fluid nutrient medium under appropriate physical and chemical conditions, then the number of viable cells per millilitre is determined periodically, and plotted, a characteristic growth curve with four phases is obtained (Fig. 4):

1. **Lag phase:** The initial number of bacterial cells remains constant. During this period, the cells adapt to their new environment. Enzymes and intermediates are formed to permit growth. $\rightarrow$ No cell division so number is constant
2. **Exponential (logarithmic) phase:** There is marked increase in cell number and its rate is accelerated exponentially with time giving a characteristic linear plot on a logarithmic scale. In this phase, the organism shows typical morphology.
3. **Stationary phase:** Exhaustion of nutrients and accumulation of toxic products cause growth to decrease. There is slow loss of cells through death which is just balanced by formation of new cells through growth and division. The number of viable bacteria remains constant. $\rightarrow$ death = replication so number is constant due to balance
4. **Decline phase:** At the end of the stationary phase, the death rate increases and exceeds the multiplication rate due to nutrient exhaustion and accumulation of toxic metabolic end products. So, the number of viable bacteria decreases.

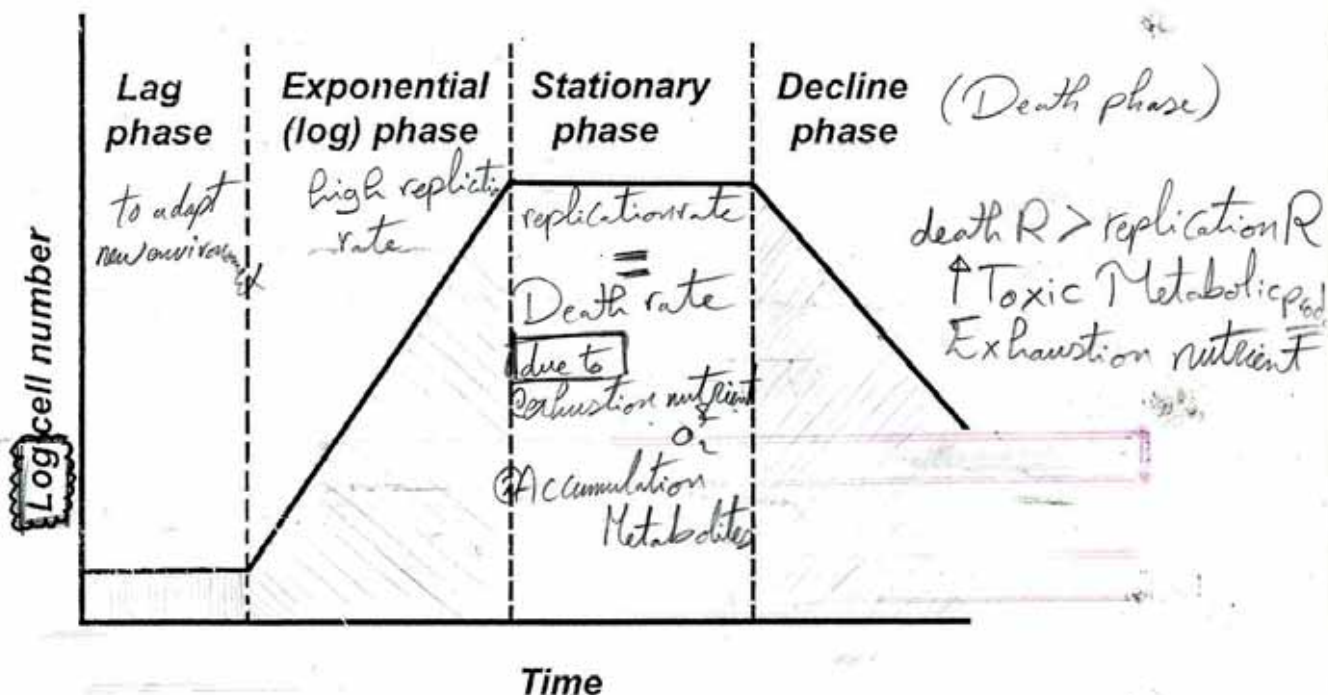


Fig. (4): Phases of bacterial growth curve.

- Give Reason :
- ① obligate anaerobes can't live in O_2 ?
 - ② Aerotolerant anaerobes can live in O_2 ?
 - ③ Anaerobes produce less ATP than

Chapter 4

Virus only infect Bacteria

BACTERIAL VIRUSES (BACTERIOPHAGES)

Bacteriophages (or phages) are viruses that parasitize bacteria i.e. the bacterial cell serves as a host for the virus.

Morphology of the Bacteriophage: (Fig. 5)

In most cases, the bacteriophage consists of:

1. **A head:** containing the nucleic acid core (usually DNA, rarely RNA) surrounded by a protein coat (capsid).
2. **A tail:** consists of a hollow core surrounded by a contractile sheath which ends in a base plate to which tail fibres are attached.

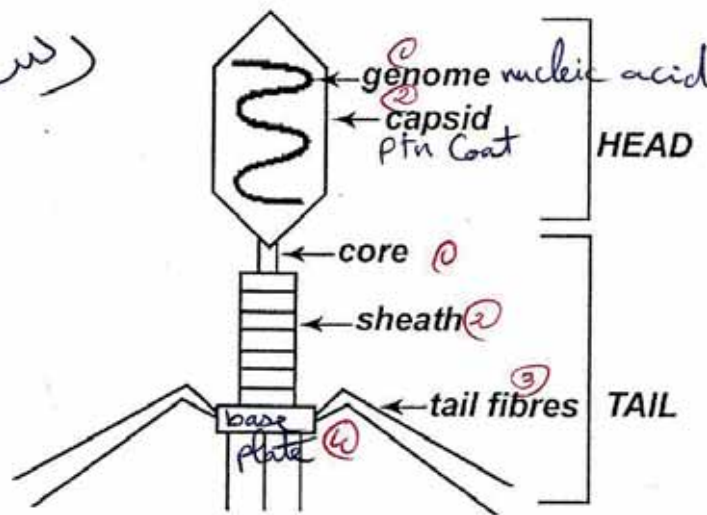


Fig. (5): Structure of a bacteriophage.

= Bacterial virus = Has no cell struct.
= No cytoplasm
= No nucleus
= No ribosome

Replication (Propagation) of Bacteriophages

Two cycles for phage replication are known:

→ Lytic cycle (vegetative)
→ Temperate cycle (Lysogenic)

A- Lytic (vegetative) cycle (Fig. 6)

It is so-called because it ends in lysis of the bacterial host cell and release of the newly formed phages. The stages of this cycle are:

1. **Adsorption:** The phage attaches, by its tail, to specific receptors on the bacterial cell. The specificity of this process determines the susceptibility of bacteria to different phages.
2. **Penetration:** The tail sheath contracts and the nucleic acid is injected into the cell. The empty head and the tail are left outside the cell.
3. **Eclipse phase:** in which no phage components are detected inside the cell. It takes a short time (minutes to hours) during which viral nucleic acid directs the host cell metabolism to synthesize the enzymes and proteins required for phage synthesis.

stages



Stages of Lytic

4. **Intracellular synthesis**: of phage nucleic acids, capsids and tails. Several hundreds of phage components are synthesized.

5. **Assembly**: The phage components aggregate to form complete phage particles which mature into typical infectious phages.

6. **Release**: The bacterial cell bursts liberating a large number of phage particles to infect new cells.

N.B.: During the lytic phage cycle, fragments of the bacterial DNA may be incorporated into the phage head. The phage can then transfer the incorporated bacterial DNA into another bacterial host **generalized transduction** (Chapter 6).

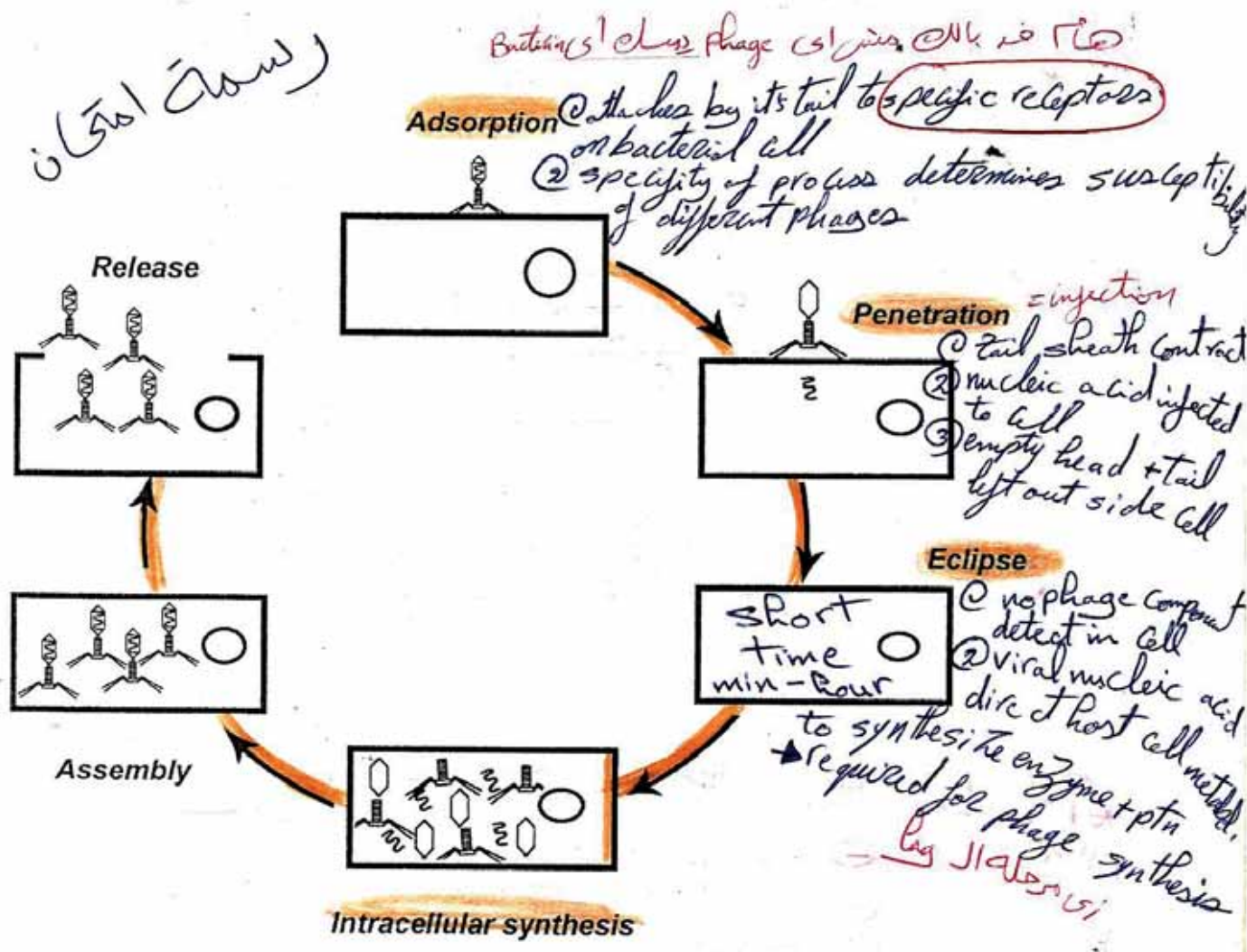


Fig. (6): The lytic cycle of bacteriophage.

B- Temperate (lysogenic) cycle

not replicate

In this cycle, the phage (temperate phage) does not replicate and lyse the bacteria but the phage DNA becomes integrated with the bacterial chromosome and divides with it to pass into daughter cells. The integrated phage genome is called "**prophage**" and the bacteria carrying it are called "**lysogenic**" bacteria.

Integrated phage DNA genome

Define = bacteria carrying

Wikipedia



The presence of the prophage in the bacterium renders it:

1. Immune to infection by another phage.
2. Lysogenic: the bacterium acquires new properties, e.g. diphtheria bacilli can produce toxin only when lysogenized. Acquisition of a new character coded for by a prophage DNA is called "lysogenic conversion", or "phage conversion". When the phage is lost from the bacterium, this new characteristic is lost.

↗️ To Jala

bacteria + Toxin character

Outcome of the temperate cycle

1. The prophage may be carried inside the bacterial cell indefinitely passing to daughter cells.
2. The prophage may be induced to detach from the bacterial chromosome and start a lytic cycle. Induction may be spontaneous or achieved by an inducer, e.g. U.V. light.

During the process of induction, the prophage may carry with it few genes of the bacterial chromosome. When it infects another bacterium, it passes this fragment to it giving it new characters. This is known as "specialized transduction" (Chapter 6).

Practical Uses of Bacteriophages

1. Phages are used as cloning vectors in recombinant DNA technology. They carry and introduce foreign DNA fragments into a host cell.
2. Phage typing: Since bacteria differ in their sensitivity to different phages, phages are used to identify and type strains of bacteria that are biochemically and antigenically indistinguishable. This phage typing is important in epidemiologic studies, e.g. to trace the source of infection in outbreaks of post-operative wound sepsis caused by Staphylococcus aureus.
3. Phages are used as research elements in some biological and genetic studies.

Cloning vector
Research element
Identify & Type strains

* Mention other methods of Bacterial typing?

Call it

- ⊖ serological typing
- ⊖ morphological typing
- ⊖ Phage typing
- ⊖ ribotyping

Chapter 5

BACTERIAL GENETICS

Genetics is the science, which defines and analyzes heredity. The unit of heredity is the **gene**, a segment of DNA that carries information for a specific biochemical or physiologic property.

The **bacterial genome** is the total set of genes present inside the bacterial cell. It comprises:

1. The **bacterial chromosome** that can encode up to **4000** separate genes necessary for bacterial growth and propagation. Additional genes may be carried on:
2. Plasmids.
3. Transposable genetic elements, and
4. Bacteriophage DNA (prophage).

plasmid
Transposable genetic element
Bacteriophage DNA

1. The Bacterial Chromosome: (Fig. 7)

Being a prokaryote, the bacterial cell lacks a nuclear membrane, instead, the DNA is concentrated in the cytoplasm as a **nucleoid**.

1 nucleoid = 1 single chromosome

The nucleoid consists of a single chromosome, which is a **circular, supercoiled, double-stranded DNA molecule**, associated at one point with a **mesosome**. This attachment plays a role in the separation of the two sister chromosomes following chromosomal replication.

① The bacterial chromosome has the **general chemical structure of DNA molecules**, i.e.:

- Each strand is formed of **regularly alternating phosphate and sugar (deoxyribose) groups**.
- A **nitrogenous base (A, G, C, or T)** is attached to the sugar group and is projecting inwards towards the other strand.
- The **two strands** are held together by **hydrogen bonds** between complementary bases (A-T) or (C-G) present at the same level.
- The **average length of the bacterial chromosome is 4000-5000 Kbp.**

② The bacterial chromosome replicates by the **semi-conservative method of DNA replication**, i.e.:

- The **two strands** are separated.
- Each strand acts as a **template to synthesize a complementary strand** through the action of the **polymerase enzyme**.

③ The bacterial chromosome follows the same rules of **gene expression and protein synthesis (i.e. transcription and translation)** as higher organisms.

* Functions of plasmid
 ① sex pilus by fertility factors which mediates conjugation = Conjugative plasmids

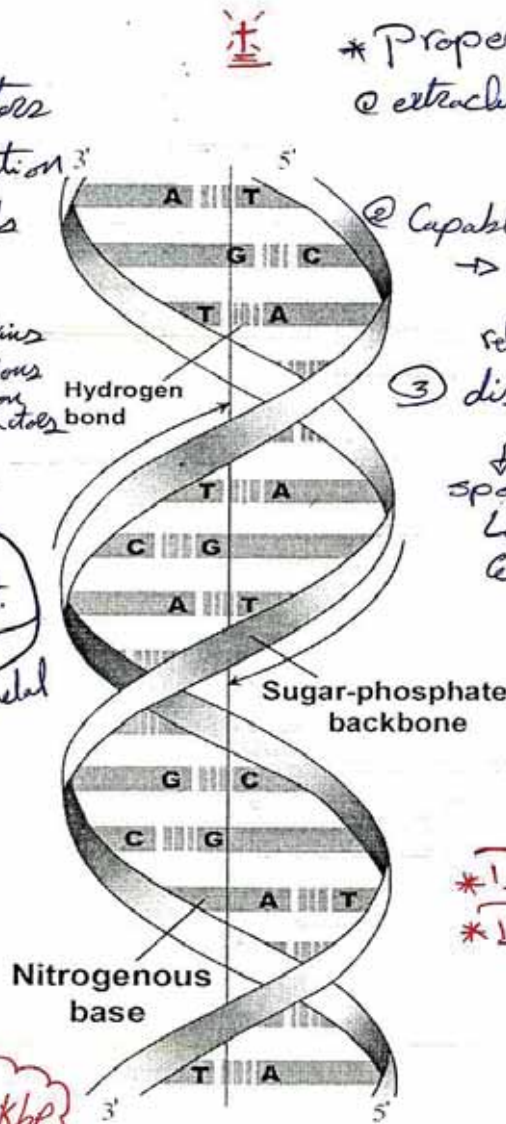
② Antibiotic resistance

③ virulence plasmids { *exotoxins*, *adhesions*, *invasions*, *toxins* }

④ Bacteriocin production substance against other strains

⑤

nitrogen fixation	sugar fermentation	Antibiotic production	H ₂ S product.
degradation aromatic compounds			Resist heavy metal



* Properties of plasmid

① extrachromosomal + circular & double stranded & smaller in size 100 kbp

② Capable of replicating → plasmid copy number
 relaxed → stringent

③ dispensable due to
 spontaneous loss in cell division
 plasmid curing + kicked off due to heat or antibiotics

* Define Bacteriocin
 * Define Bactericidal Antibiotic

MCQ

$$\text{Plasmid} = \frac{100}{5000} = \frac{1}{50} \text{ kbp}$$

Fig. (7): Structure of DNA.

2. Plasmids

→ properties

Plasmids are extra-chromosomal, circular, double-stranded DNA molecules dispersed in the cytoplasm. They are much smaller than the bacterial chromosome (from several to 100 Kbp).

Plasmids are capable of replicating independently of the bacterial chromosome. Thus, multiple copies of the same plasmid may exist in the same cell (plasmid copy number). According to the copy number, plasmids can be categorized into 2 groups: *ideal cloning vector*

1. **Relaxed replicating plasmids:** They can replicate in the absence of protein synthesis. They are usually present in 30-50 copies/cell, and are relatively small in size.

2. **Stringent plasmids:** They require protein synthesis, and are usually large and present in a few copies (1-5) per cell.

Plasmids are generally dispensable. This indicates that most plasmids encode properties that are not essential for growth, replication or survival of the host bacterium. This is evidenced by:

G.R.

(G.R.)

G.R. Plasmids are dispensables?

a- The spontaneous loss of plasmids during cell division.

b- Plasmid curing: The experimental kicking off of the plasmids using physical agents (e.g. heat) or chemical agents (e.g. antibiotics).

الکلیت تفتقر دکان شمعیت
Plasmid من

Functions (traits) exhibited by plasmids

1 Sex pilus formation: Some plasmids carry fertility (F) factors that code for the formation of a sex pilus which mediates the process of conjugation. For this reason, such plasmids are also known as conjugative plasmids (Table 1).

Defines

* G.R. Antibiotic resistance may spread rapidly bet. different bacteria?

Table (1): Comparison between conjugative and non-conjugative plasmids

	Conjugative plasmids	Non-conjugative plasmids
- Size	Large	Usually small
- Copy number	1-2 (stringent)	>30 (relaxed)
- F factors	Present	Absent
- Sex pilus formation	Yes	No
- Transfer among bacteria	by conjugation	by the help of a conjugative plasmid
- Host bacteria	Common in Gram -ve bacilli	Common in Gram +ve cocci

because R-factors usually conjugated plasmids

2 Antibiotic resistance: Some plasmids carry genes for resistance (R-factors) to one or several antimicrobial drugs. They often control the formation of enzymes capable of destroying the antimicrobial drugs, e.g. β -lactamase enzyme which determines resistance to penicillin and cephalosporins.

* R-factors are usually conjugative plasmids that can be transferred among bacteria by conjugation. This results in the rapid spread of drug-resistance among bacterial populations and the development of multiple drug-resistant bacterial strains.

3 Virulence plasmids: may code for exotoxins, adhesins or invasion factors.

4 Bacteriocin production: Bacteriocins are bactericidal substances produced by certain bacterial strains and are active against other strains of the same or closely related species, e.g. colicin E1 produced by *E. coli*.

5 Other functions include nitrogen fixation, sugar fermentation, antibiotic production, H_2S production, resistance to heavy metals and degradation of aromatic compounds.

3. Transposable Genetic Elements

These are non-replicating DNA segments (units) that are capable of inserting themselves into other DNA molecules (replicons). They are also capable of mediating their own transfer from one location to another on the same chromosome or between chromosomes and plasmids.

Transposition relies on the ability of the transposable elements to synthesize their own specific recombination enzymes (transposases or integrases).

DNA segment + Part of Chromosome

Jumping genes

is not frequently

Transposition occurs infrequently (once every 10^5 - 10^7 generations). It often occurs in a random pattern.

The insertion of a transposable element into a gene usually leads to inactivation (or disruption) of that gene.

Different classes of transposable genetic elements exist:

1. Insertion sequences (IS elements):

- They are the simplest type of transposable elements.
- They are short DNA segments (750-2000 bp).
- They encode only the enzymes necessary for:
 - a- Recombination (transposition), and
 - b- The control of the frequency of transposition.

2. Transposons:

- They are relatively complex transposable elements.
- They encode specific genes (such as antibiotic resistance), flanked by two IS elements.

3. Integrans:

- They are a specific class of transposons capable of capturing and mobilizing genes contained in mobile elements called "gene cassettes".
- Gene cassettes: are genes that can be clipped out of one integron and inserted into another (something like taking a tape recorder cassette and playing it on somebody else's tape deck).

4. Pathogenicity islands (PAI): → الترم الثاني

- Pathogenicity islands (PAI) are DNA segments (10 to 200 kb) containing mobile genetic elements inserted within the bacterial chromosome. They give the bacterium a variety of virulence characters, such as the ability to adhere to or invade host cells.
- They are usually genetically unstable. Virulence functions encoded by certain PAI are lost with a frequency that is higher than the normal rate of mutation. It is due to loss of large portions of a PAI or even the entire PAI.

4. Bacteriophage DNA

The DNA of the temperate bacteriophage that is integrated in the chromosome of a lysogenic bacterial cell (i.e. the prophage) is considered as a part of the genome of such bacteria (see chapter 4).

Chapter 6

change in Bacterial characters

BACTERIAL VARIATION

Phenotypic

Genotypic

Bacterial variations are changes in the bacterial characters. They may be phenotypic or genotypic (Table 2).

Table (2): Comparison between phenotypic and genotypic variations:

Define Phenotypic variation	Define Genotypic variation
- It occurs in response to <u>changes in the environmental conditions without change in the genetic constitution</u>	- It occurs as a result of a <u>change in the underlying genetic constitution</u>
- Reversible (transient)	- Irreversible (permanent)
- Not-heritable	- Heritable
- Examples: 1. L-forms of bacteria 2. Loss of flagella upon exposure to phenol	- It occurs through: 1. Mutation 2. Gene transfer: - a- Transformation - b- Transduction - c- Conjugation

*after removing penicillin become with cell wall again

* Genotypic variation *

Mutation

It results from a change in the nucleotide sequence of DNA that may occur spontaneously as a replication error (at a rate of once every 10^6 - 10^7 cells); or may be induced by radiation or chemical agents (at a higher rate of once every 10^3 - 10^4 cells).

Mutation can be classified according to nucleotide substitution, insertion or deletion into:

1. **Single-base (point) mutations** involve the replacement (substitution) of a single nucleotide in the coding sequence. This may result in:

a- **Same sense (silent) mutations**: occur when the resulting base triplet (codon) codes for the same amino acid as the original triplet.

b- **Missense mutations**: occur when the mutant base changes the coding sequence so that a different amino acid is produced. The resulting protein may be functioning or not, depending on the importance of the area affected by the mutation.

2. **Frame-shift mutations** occur when a nucleotide is inserted into, or deleted from the coding sequence, resulting in a shift of the reading frame, e.g. insertion of a transposable element.

Induced mutations may be used to manipulate viral genomes for vaccine production and gene therapy.

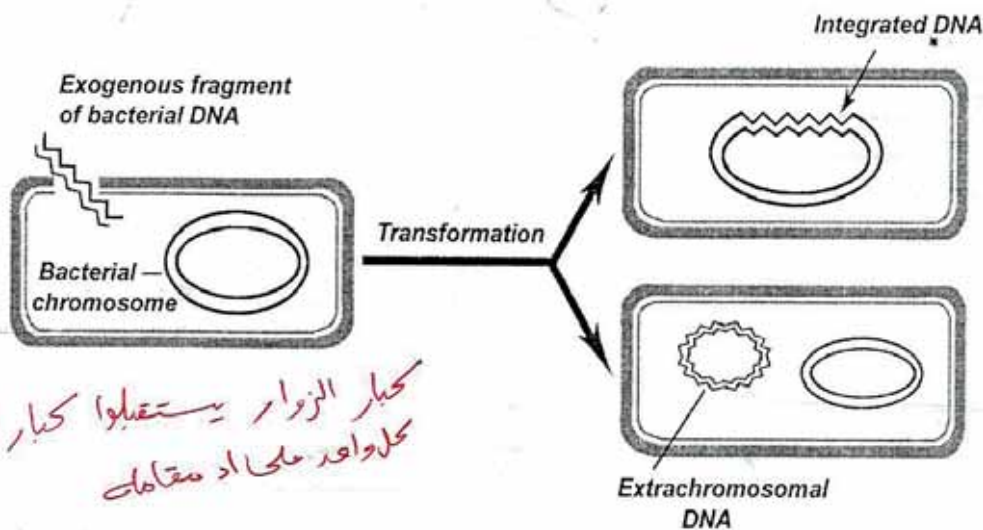
Gene Transfer

There are 3 methods for gene transfer among bacteria:

1. Transformation: (Fig. 8)

Dying bacteria release DNA which can be taken up by other bacteria. Such DNA may be chromosomal or plasmid in origin, and may carry genes that "transform" the recipient bacterium.

The transforming DNA may become integrated with the bacterial chromosome or re-established extrachromosomally in the recipient cell (Fig. 8).



كبار الزوار يستقبلوا كبار الزوار
كل واحد على اد مقامه

Fig. (8): Gene transfer by transformation.

Transformation depends on competence, which is the ability of the recipient bacterial cell to take up DNA. Competence depends on the presence of proteins in the cell membrane that have a special affinity to bind DNA and transport it into the cytoplasm.

Artificial competence can be induced during recombinant DNA techniques by treating the recipient bacteria with calcium chloride, which alters cell membrane permeability, enabling the uptake of DNA.

2. Transduction

It is the transfer of DNA from one cell to another by means of a bacteriophage. vector
There are 2 types of transduction:

a- Generalized transduction:

During the lytic phage cycle, the bacterial DNA is fragmented and any fragment of DNA (whether chromosomal or plasmid) may be incorporated into the phage head. The phage particle can then transfer the incorporated bacterial DNA into another bacterial host.

b- Specialized transduction:

It takes place when a prophage contained in a lysogenized bacterial cell is induced to detach. Such prophage may carry with it the adjacent piece of the chromosomal DNA and transfer it to another bacterial cell.



Table (3): Comparison between generalized and specialized transduction:

	Generalized transduction	Specialized transduction
-Type of phage	Lytic (virulent) phage	Temperate (lysogenic) phage
-Replication cycle	Lytic cycle	Lysogenic cycle
-The transferred DNA fragments	Any piece of bacterial DNA (chromosomal or plasmid) <i>= Random piece</i>	A specific piece of chromosomal DNA <u>adjacent</u> to the site of insertion of the prophage

3. Conjugation: (Fig. 9)

Most frequent ✓✓ = 2 intact bacteria donor + recipient

It is the most frequently observed mechanism of DNA transfer. It involves 2 cell types: donors (F^+) which possess the fertility (F) factor, and recipients (F^-) which lack the F factor.

The F factor carries the genes for the synthesis of the sex pilus which acts as a conjugation tube between the donor and recipient bacterial cells. The 2 DNA strands of the F factor are then separated, and one strand is transferred from the donor to the recipient cell. Each strand forms a complementary strand, thus, the recipient cell acquires a copy of the F plasmid and becomes an F^+ cell.

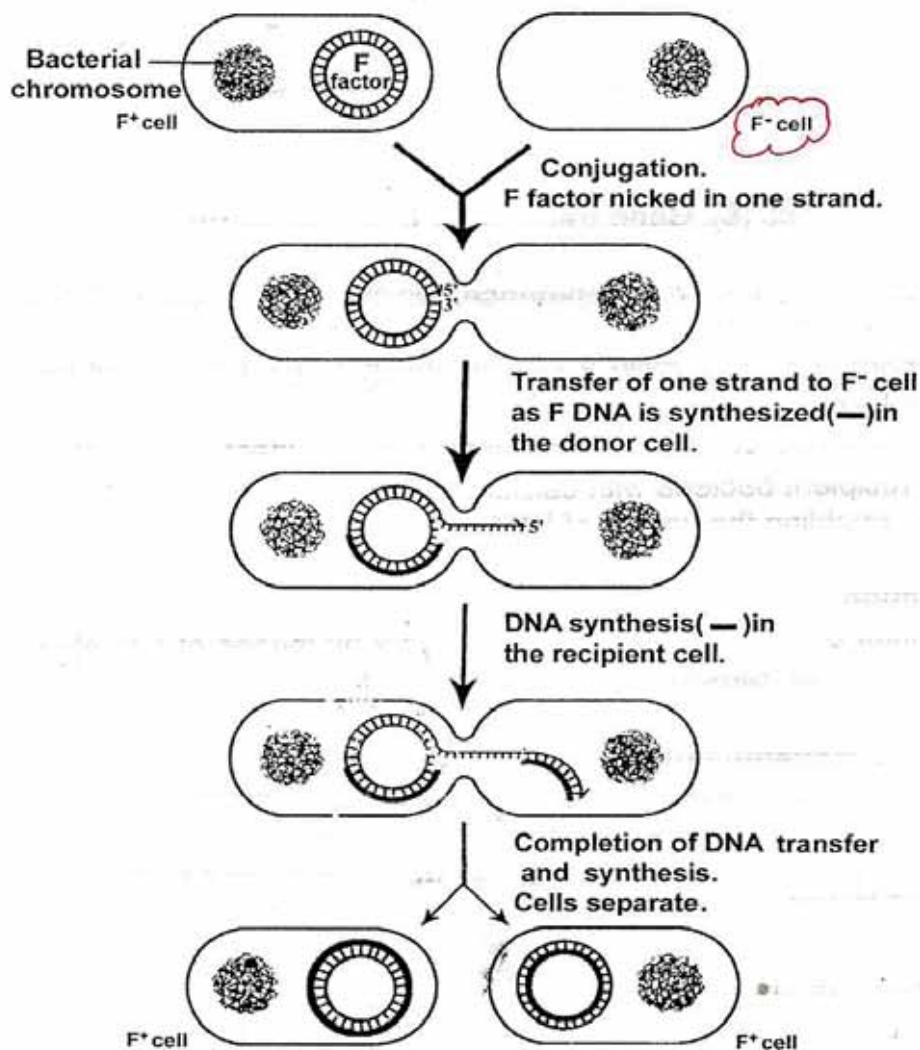


Fig. (9): Gene transfer by conjugation.

Chapter 7

GENETIC RECOMBINATION (GENE CLONING)

The recent technology which is called genetic recombination, recombinant DNA technology, genetic engineering or gene cloning involves the *in vitro* modification and recombination of genetic material from different organisms to construct new gene combinations.

Recombinant DNA Technique: (Fig. 10)

A typical cloning experiment requires:

1. DNA of interest, sometimes called foreign, passenger, target or insert DNA. It is obtained by extracting the DNA from a given source and cleaving such DNA by a specific restriction endonuclease enzyme.
2. A cloning vector; usually derived from an extrachromosomal replicon such as plasmids, cosmids, bacteriophages, or animal viruses.
3. Restriction endonuclease enzymes to produce site-specific scissions in the DNA molecules.
4. DNA ligase to anneal the complementary ends of the vector and target DNA, thus producing a recombinant DNA molecule.
5. A prokaryotic or eukaryotic cell to serve as the biological host.

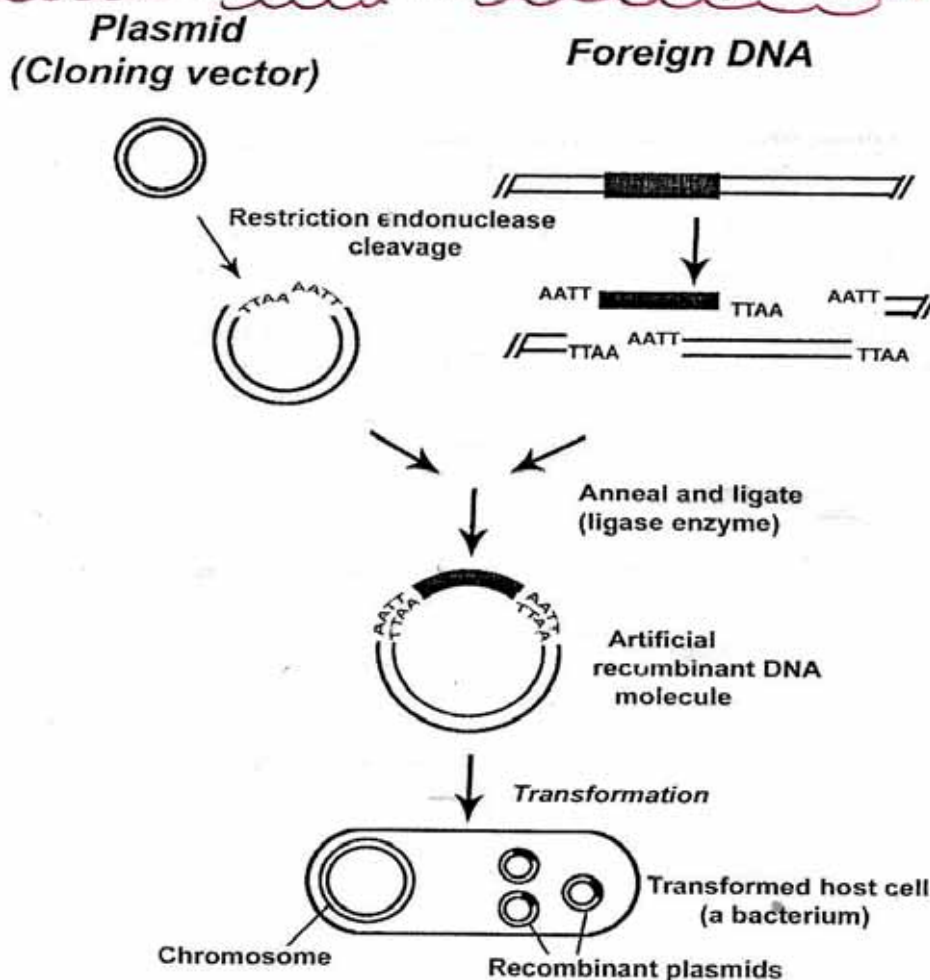


Fig. (10): The recombinant DNA technique.

I. Cloning Vectors

Define = carrier or vehicles

These are vehicles used to carry and introduce foreign DNA fragments into a host cell. An ideal cloning vector must fulfil certain requirements:

1. It must be as small as possible to contribute as little as possible to the overall size of the recombinant molecule.
2. It must be well characterized regarding gene location and nucleotide sequence.
3. It must possess a single cleavage site for at least one restriction endonuclease, at which foreign DNA can be inserted.
4. It must be capable of autonomous replication within the host cell so that amplification and isolation of the recombinant DNA molecule can be achieved.
5. It must carry a selectable marker (e.g. antibiotic resistance gene) so that the host cells transformed with the vector can be distinguished from non-transformed cells.

G.R.

G.R. relaxed plasmid is ideal cloning vector?

At present, four types of cloning vectors are in common use:

1. **Plasmids:** Relaxed plasmids are among the best cloning vectors as they fulfil most of the requirements for ideal vectors. Recombinant plasmids can be introduced into the host cells by the process of transformation.

*= 10-1000 copies
= replicates
= many copies
= non conjugated
= small size*

2. **Bacteriophages:** Some bacteriophages (e.g. phage M13 and lambda phage of *E. coli*) are used extensively as prokaryotic cloning vectors. They can introduce foreign DNA into their bacterial host by the process of transduction.

Define + Importance?

3. **Cosmids:** These are artificially constructed cloning vectors. They are formed of plasmids and the cohesive end (COS) of the lambda phage DNA. They are useful in cloning large DNA fragments of up to 40 Kbp by the process of transduction.

4. **Animal viruses:** Viral genomes have been manipulated to serve as cloning vectors for eukaryotic host cells. The most commonly used viruses are retroviruses, adenoviruses and vaccinia virus. Such viruses are used as gene delivery vehicles in the trials of gene therapy.

II. Restriction Endonuclease Enzymes

Define

These are enzymes that recognize specific nucleotide sequences within DNA molecules and catalyze the cleavage of both strands of DNA at internal positions within these sequences.

So called endonucleases

At present, about 500 different restriction endonucleases have been identified, most of which are obtained from a wide variety of bacteria and a few are obtained from fungi and algae. These enzymes are named according to the organism from which they are derived e.g. EcoRI is derived from *E. coli*.

The bacterial own DNA is methylated in a specific manner to be protected from cleavage by the restriction endonucleases it produces. The activity of the enzymes is, thus, restricted to unmodified foreign DNA, hence they are called restriction enzymes.

III. Ligation of DNA Fragments

The construction of recombinant DNA molecules (i.e. the cloning vector and the integrated foreign DNA) is dependent on the ability to covalently seal single-strand breaks in DNA. This process is accomplished by the DNA ligase enzyme.

The DNA ligase more commonly used in molecular cloning is the T₄ DNA ligase derived from *E.coli* infected with phage T₄. It has the ability to join together sticky as well as blunt-ended DNA fragments by catalyzing the formation of phosphodiester bonds between the 3'-hydroxyl and the 5'-phosphate ends of the fragments.

IV. Host Organisms

Microorganisms commonly used as hosts for cloning experiments include the Gram-negative rod *E.coli*, the Gram-positive spore-forming rod *Bacillus subtilis*, and the yeast *Saccharomyces cerevisiae*. Ideally, strains used for this purpose should be free of any restriction enzyme activity that might degrade foreign DNA. Further, it is more advantageous that the nucleic acid of the host be easily separable from that of the cloning vector by physical or chemical means.

Applications of Recombinant DNA Technology

1. Extensive study of gene structure and function which enables mapping of the microbial genomes.
2. Production of biological products of medical importance, e.g. hormones (such as insulin), interferons, interleukins, monoclonal antibodies...etc.
3. Production of recombinant vaccines, e.g. hepatitis B vaccine. The gene coding for hepatitis B surface antigen (HbsAg) is cloned in yeast cells where it is expressed. The gene product (i.e. HbsAg) is extracted from the yeast cells, purified and used for immunization.
4. Preparation of genetic probes; which are short single-stranded DNA or RNA fragments, used for the diagnosis of infectious as well as genetic diseases.
5. Gene therapy; where viruses (e.g. retroviruses or adenoviruses) can be used as gene delivery vehicles to replace defective genes with new functioning genes, e.g. in case of immunodeficiency or diabetes mellitus.

enumerate 4 essential component in all bacteria

- ① Cytoplasmic memb. ~~transport~~ + Biosynthesis
 - ② nucleoid replicate + gene expression
 - ③ Ribosome
 - ④ Cell wall shape + stain + cytoplasm memb. protect + division
- + energy product
+ reprod + chemotaxis + excretion

It is more blessed to give than to receive.
Antimicrobial synthesized to treat by kill microorganism

Antibiotic 2nd metabolite of microorganism

Chapter 8

ANTIMICROBIAL CHEMOTHERAPY

Antimicrobial chemotherapeutic agents: are chemically synthesized substances that are used to treat infectious diseases by killing or inhibiting the growth (or multiplication) of microorganisms.

Antibiotics are low-molecular weight antimicrobial substances that are produced as secondary metabolites by certain groups of microorganisms, especially Streptomyces, Bacillus, and a few moulds (Penicillium and Cephalosporium).

Although their original source was a microorganism, some antibiotics are currently made synthetically (synthetic antibiotics). Chemical modification of certain antibiotics, to achieve the desired properties, has been a prominent method of new drug development (semisynthetic antibiotics).

Bacteriostatic agent: is an antimicrobial agent that is capable of inhibiting bacterial multiplication. Multiplication resumes upon removal of the agent.

Bactericidal agent is an antimicrobial agent that is capable of killing bacteria. Multiplication can not be resumed.

Selective toxicity is the ability of an antimicrobial agent to harm a pathogen without harming the host. It may be a function of a specific receptor (or target) for the drug found in the microbe but not in the human body (e.g. peptidoglycan), or it may depend on the inhibition of a biochemical event essential for the organism but not for the host.

Spectrum of activity the range of microorganisms that are affected by a certain antibiotic is expressed as its spectrum of action. Antibiotics which kill or inhibit the growth of a wide range of Gram-positive and Gram-negative bacteria are said to be broad spectrum. If effective mainly against either Gram-positive or Gram-negative bacteria, they are narrow spectrum. If effective against a single organism or disease, they are referred to as limited spectrum.

Mechanisms of Action of Antimicrobial Agents

A- Inhibition of bacterial cell wall synthesis

Agents acting by this mechanism include:

1. β -lactam antibiotics: e.g. penicillins, cephalosporins, and others.
2. Glycopeptides: e.g. vancomycin, and teicoplanin.
3. Cycloserine and bacitracin.

These antibiotics are bactericidal with minimal tissue toxicity.

The β -lactam drugs inhibit the last steps of peptidoglycan synthesis. This inhibition is initiated by binding of the drug to certain cell receptors known as penicillin-binding proteins (PBPs). On the other hand, glycopeptides and cycloserine inhibit early steps in the biosynthesis of peptidoglycan, which occur inside the cytoplasmic membrane. Therefore, the mechanism of resistance to β -lactam antibiotics is different from that for the other groups. Subsequently, vancomycin could be used successfully in infections caused by β -lactam resistant staphylococci.

G.R. Bactericidal & low toxicity to host? bec. attach cell wall essential for... & osmotic lysis

+ selective toxicity against organism not host

if bacteria resist penicillin can we use vancomycin & why? because different stages & different sites actions

B- Interference with the cell membrane function

Some agents disrupt the cytoplasmic membrane and interfere with its function. These include:

1. Antibacterial agents: e.g. polymyxin and colistin.
2. Antifungal agents: e.g. amphotericin B, nystatin and imidazoles.

These agents are microbicidal. They are highly toxic (as) they have narrow margin of selective toxicity.

* Give reason? bec. narrow margin selective toxicity & similarity to cell memb. of host

C- Inhibition of bacterial protein synthesis

Bacteria have 70S ribosomes (with 30S and 50S subunits) whereas mammalian cells have 80S ribosomes (40S and 60S subunits). This difference makes bacterial ribosomes a selective target for antimicrobials:

1. Agents acting on the 30S ribosomal subunit: e.g. tetracycline and aminoglycosides (gentamicin, amikacin, streptomycin).
2. Agents acting on the 50S ribosomal subunit: e.g. macrolides (erythromycin, azithromycin), lincomycins (clindamycin), streptogramins, linezolid, chloramphenicol and fusidic acid.

GR. bec. Bacteria Ribosome selective target?

dis. 8/17

D- Inhibition of bacterial nucleic acid synthesis

This may occur by:

1. Inhibition of RNA synthesis through the strong binding to DNA-dependent RNA polymerase: e.g. rifampin.
2. Inhibition of DNA synthesis through blocking DNA gyrase: e.g. quinolones and novobiocin.
3. Inhibition of dihydrofolic acid reductase leading to inhibition of folic acid synthesis. The latter is important for purine synthesis and, consequently, nucleic acid formation. Examples of these antimicrobials include trimethoprim and pyrimethamine.
4. Inhibition of folic acid synthesis by competitive antagonism e.g. sulphonamides. For many organisms, para-amino benzoic acid (PABA) is essential for the synthesis of folic acid. Sulphonamides are structural analogues of PABA. They compete with PABA for the active centre of the enzyme involved in folic acid synthesis. As a result, nonfunctional analogues of folic acid are formed and nucleic acid synthesis is inhibited.

Choice of an Antimicrobial Agent for Therapy

The following are guidelines that can be followed for proper antibiotic use:

1. Select an antibiotic that is able to penetrate to the site of infection and achieve effective concentration, e.g. certain drugs are able to pass the blood-brain barrier, others are highly concentrated in urine.
2. Identify the nature of the infection whether bacterial, viral, fungal, or parasitic. A common mistake is to give an antibacterial agent for a viral infection.
3. Give the appropriate dose of the antibiotic for the proper duration. Inadequate dosage or undue prolonged therapy may result in drug toxicity and antibiotic resistance.
4. Choose as narrow an antibiotic spectrum as you can. When you get the results of culture and susceptibility, revise your treatment to 'narrow-down'

G.R. 3. G.R. 4.

* Give reason viral infection not treated by Antibiotics?

Write complete



the spectrum as far as possible. The use of broad spectrum antibiotics is likely to faster induce resistance to antibiotics and may be complicated by superinfection.

5. Know the potential of the drug to produce toxicity: Some drugs known to be of low toxicity will exert high toxicity if they accumulate in the blood due to liver or kidney dysfunction. Use antibiotics that are only safe for the pregnant and lactating women and for infants and children.
6. Choose bactericidal rather than bacteriostatic antibiotics.

because it only inhibit replication :: after stop re replicate

Microbial Susceptibilities to Antimicrobial Agents

Microorganisms vary in their susceptibility to different chemotherapeutic agents, and susceptibilities can change over time. Ideally, the appropriate antibiotic to treat any particular infection should be determined *in vitro* before any antibiotic is given.

The *in vivo* activity of an antimicrobial agent is not always the same as its *in vitro* susceptibility because it involves many host factors that are not tested *in vitro*.

The activity of an antimicrobial agent against an organism is dependent on its concentration. Some idea of the effectiveness of a chemotherapeutic agent can be obtained from determining the minimal inhibitory concentration (MIC).

The MIC is defined as the lowest concentration of a drug that prevents growth of a test organism.

The MIC forms the basis for susceptibility and determining breakpoints. The breakpoint of an antimicrobial agent is the concentration that can be achieved in the serum with optimal dose. Organisms with MICs at or below the breakpoint are considered susceptible. On the other hand, organisms with MICs above the breakpoint are considered resistant.

The routine *in vitro* susceptibility testing can be done by one of the following methods (Chapter 23, Vol. III):

1. Disc diffusion method.
2. Dilution method such as tube broth dilution.
3. Gradient diffusion (E test) method.

+ host factors

Empiric Therapy

The empiric therapy is a "best guess" procedure based upon a provisional diagnosis made by the physician that a patient has a bacterial infection which requires treatment. Depending on the type of infection, there will be a short list of bacteria most likely to be causing that infection. Depending on the type of bacteria, there will be an antibiotic most likely to successfully treat that infection. "Best guess" treatment is not always successful or appropriate as many bacteria have unpredictable susceptibilities to antimicrobial agents.

Indications:

1. In seriously ill patients empiric therapy should be started without delay but after collecting specimens for culture.
2. In closed lesions, where there is no available sample.

Define & what relation bet MIC & break point?
if MIC = 10 µgm
breakp. = 12 µgm
∴ MIC can act



Combined Therapy

The ideal rule in antimicrobial therapy is **mono-therapy** which means choosing **one** drug effective against a particular organism. However, there are conditions which necessitate the use of more than one antibiotic in order to achieve a successful clinical response.

Possible indications

1. Severely ill patients suspected of having serious infections, e.g.:
 - Bacterial meningitis.
 - Sepsis in immunocompromised patients caused by *Pseudomonas aeruginosa*, *Klebsiella* spp., *Enterobacter* spp. or *S. aureus*.
2. Febrile neutropenia.
3. To delay the emergence of drug-resistant mutants e.g. in treatment of T.B.
4. To achieve bactericidal action through synergistic effect e.g. in enterococcal endocarditis.
5. Mixed infections e.g. infections following massive trauma.

Effects of combined therapy

1. Synergistic effect ($1+1 \Rightarrow 2$): The combined action is significantly greater than the sum of both effects, e.g.:
 - Vancomycin + gentamicin in treatment of methicillin-resistant staphylococci.
 - Sulfamethoxazole + trimethoprim (cotrimoxazole) in treatment of shigellosis.
2. Antagonistic effect ($1+1 < 1$): The combined action is less than that of the more effective agent when used alone, e.g.:
 Penicillin + chloramphenicol in treatment of meningitis.
3. Indifference ($1+1 = 1$): The combined action is no greater than that of the more effective agent when used alone, e.g.:
 Cefepime + vancomycin, or clindamycin + vancomycin.
4. Addition ($1+1 = 2$): The combined action is equivalent to the sum of the actions of each drug when used alone.

Complications of Chemotherapy

1. Toxicity: may be dose-dependent or independent, e.g.:
 - Tetracycline may cause staining of teeth in infants.
 - Streptomycin may affect the 8th cranial nerve leading to vestibular dysfunction.
 - Aminoglycosides may cause nephrotoxicity.
 - Chloramphenicol can cause bone marrow depression.
2. Allergy (hypersensitivity): usually not dose-dependent, e.g.:
 - Penicillins may cause urticaria, anaphylactic shock or serum sickness.
 - Local application of sulphonamides may result in contact dermatitis.

↳ Type IV

3. Emergence of resistant strains: The abuse of antibiotics (low dosage, interrupted course, no real indication, and improper choice) encourages the emergence of resistant mutants. These mutants will overgrow and replace the originally susceptible bacteria. It is recommended that in vitro susceptibility testing should be performed to guide the selection of antibacterial drugs.

4. Superinfection: It occurs as a result of outgrowth of resistant members of normal flora when the sensitive ones are eradicated during antibiotic therapy e.g.:

- Pseudomembranous colitis caused by outgrowth of *Clostridium difficile*,
- Oral thrush caused by overgrowth of the yeast *Candida*.

Resistance to Antimicrobial Agents

7/10/2020

2

Antibiotic resistance is a global problem faced today in the treatment of infectious diseases. Resistance to antibiotics is more prevalent in hospitals especially intensive care units due to the higher antibiotic use. Resistance to antimicrobial agents is of two categories either intrinsic or acquired.



Intrinsic (inherent or natural) resistance

This type of resistance refers to bacteria that are insensitive in their natural state, to an antibiotic without the acquisition of resistance factors. It is consistent and can be expected once the organism is known. Intrinsic resistance occurs in the following situations:

① *Streptomyces* are protected from the antibiotics they produce.

② Gram-negative cell membrane has pores too small to allow large antibiotic molecules, e.g. nafcillin, to penetrate.

G-ve pores

3. An organism lacks the target or receptor for the antibiotic as in the case of resistance of *Enterococcus* species to cephalosporins. or *Mycoplasma*

Acquired resistance

It results from altered bacterial physiology and structure due to changes in the genome of the organism. It is inconsistent and unpredictable. The unpredictable nature of this resistance is the 1st reason why laboratory methods to detect resistance are necessary.

Acquired resistance mechanisms are driven by two genetic processes in bacteria:

(1) **Mutation and selection** (sometimes referred to as vertical evolution): Exposure of an organism to an antibiotic exerts a selective pressure on the organism and leads to mutation. The more frequent the exposure to the antibiotic the greater the potential resistance.

Vertical

(2) **Exchange of genes** between strains and species (sometimes called horizontal evolution). Resistance genes can be encoded on plasmids, phages, transposons and integrons (see chapter 5).

Horizontal

Mechanisms of acquired resistance

Bacteria have the ability to use one or more of the following mechanisms:

A) Reduction of the intracellular concentration of the antibiotic by:

a. Decrease in influx of antibiotic through: ↓ influx

- Reduction of permeability of the outer membrane by modification or loss of porin (a hollow membrane protein) required for entry of the antibiotic molecules.

↑ efflux pumps

b. Efflux pumps: The antibiotic is pumped out across the cytoplasmic membrane faster than it can diffuse in, so the concentration of antibiotic remains too low to be effective.

B) Inactivation of the antibiotic, e.g.:

- Production of β -lactamases leads to hydrolysis of the β -lactam ring, thus inactivating penicillins and cephalosporins.
- Production of acetyl transferase results in chloramphenicol resistance.
- Production of aminoglycosides-modifying enzymes.

C) Target modification: Modification of the target site for the antibiotic results in a reduced affinity for its receptor:

- Modification of the penicillin-binding proteins (PBPs) is a primary mode of resistance to β -lactam antibiotics in methicillin-resistant *S. aureus* (MRSA).
- Alteration of the 50S ribosomal subunit reduces the affinity of macrolides linezolid and streptogramins for the ribosome.
- Alteration of the 30S ribosomal subunit reduces the affinity of aminoglycosides for the ribosome.

D) Target elimination by developing new metabolic pathways: These bacteria have the ability to create new metabolic pathways that bypass the original target, e.g. resistance to trimethoprim.

E) Target overproduction: This may be the mechanism used by *S. aureus* strains with intermediate susceptibility to vancomycin (VISA)

Antimicrobial Chemoprophylaxis

Chemoprophylaxis is the administration of an effective antimicrobial agent to prevent (rather than) to treat infection with a certain microbe, thus preventing development of a disease. Examples:

- Long acting penicillin (or erythromycin):** is given to rheumatic patients to prevent reinfection with *S. pyogenes*. & Rheumatic fever
- Rifampicin:** is given to close contacts of meningococcal meningitis for 2 days to prevent meningitis.
- Penicillin or erythromycin:** is given to individuals with abnormal heart valves prior to dental procedures to prevent endocarditis.
- Preoperative** in some surgical operations.

to prevent normal flora
Like in GI Surgery

③ Injection Control

Chapter 9

DISINFECTION AND STERILIZATION

relative term

absolute term
No degree

Definition and principles of terms

Sterilization: validated process used to render a product free of all forms of viable microorganisms including all bacterial spores. Steam under pressure, hydrogen peroxide gas plasma, ethylene oxide gas and dry heat are the main validated sterilization processes for use in the healthcare facilities. Sterilization is essential for culture media, and critical items intended to enter the vascular system and sterile tissues such as vascular catheters and surgical instruments.

Disinfection: It is a process that eliminates most, if not all, pathogenic microorganisms except spores. Thus unlike sterilization, disinfection is not sporicidal. Disinfection is required for devices or equipment that do not penetrate tissues but used in contact with the skin (e.g., stethoscope diaphragm swabbed with 70% alcohol) or mucous membranes (e.g., immersion of endoscope in 2% ortho-phthalaldehyde (OPA) disinfectant for 12 minutes).

Disinfectant: usually a chemical agent (but sometimes a physical agent) that achieves disinfection. It refers to substances applied to inanimate objects.

Antiseptic: a chemical disinfectant which can be safely applied to skin and mucous membranes but not suitable for systemic administration. The term is used especially for preparations applied topically to living tissue (e.g., 70% isopropyl alcohol to prepare skin for injection, preoperative skin preparation with alcohol-based iodine compound in surgical operations).

Germicide: Agent that destroys microorganisms; may be virucide, bactericide, fungicide, sporicide and tuberculocide indicating the microorganisms the germicide kills. The term germicide includes both antiseptics and disinfectants.

* Antiseptics are germicides applied to living tissue and skin; disinfectants applied only to inanimate objects.

Sterilant: chemical germicide that achieves sterilization

Disinfectants may be categorized into 3 levels: High, intermediate and low:

- High level disinfectant:** Germicide that kills all microbial pathogens except large numbers of bacterial spores. Examples include OPA for endoscopes, hydrogen peroxide for contact lens, chlorine for blood spills.
- Intermediate level disinfectant:** Germicide that kills all microbial pathogens except bacterial spores. Examples include isopropyl alcohol and iodophors.
- Low level disinfectant:** Germicide that kills most vegetative bacteria (except tubercle bacilli) and lipid-enveloped and medium-sized viruses such as human immunodeficiency virus and hepatitis B virus e.g., quaternary ammonium compounds for disinfection of floors and food preparation areas.



Gross
بالجودة

→ precede disinfection & sterilization

Cleaning (or precleaning): removal of foreign material (organic or inorganic contaminants) from medical devices as part of decontamination process. It is usually done with water and soap, detergents or enzymatic products. Cleaning must always precede disinfection and sterilization.

Decontamination: reduction of pathogenic microorganisms to a level at which items are safe to handle. Decontamination includes sterilization and all disinfection levels.

MAIN METHODS OF DISINFECTION

1. **Chemical disinfectants** (see above).
2. **Boiling water:** Boiling (100°C) for 20 minutes achieves high disinfection. It can be useful in emergencies if sterilizer is not available.
3. **Pasteurization:** Pasteurization of milk by heating at 63°C for 30 min. or at 72°C for 20 sec., followed by rapid cooling, destroys important pathogens e.g. *Mycobacterium tuberculosis*, *Brucella*, *Salmonella* and *Coxiella burnetti*.
4. **Thermal disinfection by hot water** can be performed in special washing machines e.g. for linen in hospital laundry, dishes and devices which cannot withstand higher temperature.
5. **Ultraviolet radiation (UV):** UV can be artificially produced by mercury lamps. UV rays have weak penetration power and is used only for air and surface disinfection, e.g., laboratory safety cabinets.

METHODS OF STERILIZATION

I. Steam sterilization: It is the most safe and commonly used sterilization method (see below).

II. Low temperature sterilization methods:

1. Hydrogen peroxide gas plasma Safe

- Gas plasmas have been referred to as the fourth state of matter (i.e., liquids, solids, gases, and gas plasmas). Gas plasmas are generated in an enclosed chamber under deep vacuum using radio frequency or microwave energy to excite the gas molecules and produce charged particles, many of which are in the form of free radicals.
- The free radicals interact with essential cell components (e.g., enzymes, nucleic acids) and thereby disrupt the metabolism of microorganisms, in addition to the direct inactivation by hydrogen peroxide.

@ by CPa by
 @ peracetic acid
 Endoscope

- Total time of sterilization cycle is about 50 minutes.
- Medical materials and devices that cannot tolerate high temperatures and humidity, such as some plastics, electrical devices, and corrosion-susceptible metal alloys, can be sterilized by this method.
- *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*) spores are used as a biological indicator to monitor efficiency of the sterilization process.

2. Ethylene oxide gas sterilization

- Exposure time is long and varies from 3 to 6 hours.
- The method is expensive with probable toxicity.
- It can be used for instruments that cannot be subjected to steam.
- *Bacillus atrophaeus* (formerly *B subtilis*) spores are used as a biological indicator.

3. Peracetic acid sterilization: used to sterilize medical, surgical, and dental instruments (e.g., endoscopes, arthroscopes). Peracetic acid denatures proteins, disrupts cell wall, and oxidizes proteins and enzymes of microbes.

III. Dry heat sterilization, includes the following forms:

1. **Incineration:** is particularly applicable for dead animal bodies, infectious hospital waste such as used surgical dressings, needles....etc.
2. **Red heat:** Inoculating wires, loops and points of forceps are sterilized by holding them in the flame until they are red hot.
3. **Dry Heat Sterilizers or hot air ovens**
 - The method employs dry hot air as the sterilizing agent.
 - The most common time-temperature relationships are 170°C for 60 minutes, 160°C for 120 minutes, and 150°C for 150 minutes.
 - *Bacillus atrophaeus* spores should be used as a biological indicator.
 - Mode of action, killing is due to oxidation of the microbial cell constituents
 - This method is used for materials that might be damaged by moist heat (e.g., powders, petroleum products, sharp instruments).
 - The advantages for dry heat include the following: it is nontoxic, relatively inexpensive, and it is noncorrosive for metal and sharp instruments.
 - The disadvantages are the slow rate of heat penetration, and time-consuming and the high temperatures are not suitable for most materials.

IV. Other sterilization methods

1. **Ionizing radiation:** Sterilization by ionizing radiation can be obtained by cobalt 60 gamma rays or electron accelerators (β -rays). Ionizing radiation has a high penetrating power and is, therefore, used for sterilization of prepacked heat-sensitive items such as bone grafts, surgical sutures, disposable plastic syringes, gloves, catheters and plastic Petri dishes. *Bacillus pumilus* spores are used as a biological indicator to monitor efficiency of radiation sterilization.

قبل الاستعمال
 disposable plastic syringes

مقالات
 disposable needles

2. Filtration:

- A process used to remove bacteria from thermolabile pharmaceutical fluids (antibiotic solutions, hormones, vitamins) that cannot be purified by any other means. Fluids can be rendered free of bacteria by passage through bacterial membrane filters with pore size as small as $0.22 \mu\text{m}$.
- Filters can also be used to remove microorganisms from air supplied to critical areas such as operating rooms, drug factories and laboratory biosafety cabinets. Such filters are known as high efficiency particulate air (HEPA) filters which can provide sterile air at the filter face.
- The endopigment producing *Serratia marcescens* may be used to test the efficiency of bacterial membrane filters, while spores of the fungus *Aspergillus* may be used to test the efficiency of HEPA filters.

3. Microwaves:

Microwaves are used in medicine for disinfection of soft contact lenses, dental instruments, dentures, milk, and urinary catheters. The microwaves produce friction of water molecules in an alternating electrical field leading to generation of lethal heat. Process has not been validated.

4. Vaporized Hydrogen Peroxide (VHP).

5. **Ozone:** A sterilization process which uses ozone as the sterilant. Ozone has been used for years as a drinking water disinfectant. Ozone (O_3) consists of O_2 with a loosely bonded third oxygen atom that makes ozone a powerful oxidant that destroys microorganisms.

6. **Formaldehyde Steam:** Low-temperature sterilization method that involves use of formalin, which is vaporized into a formaldehyde gas admitted into the sterilization chamber. The method may be used in healthcare facilities to sterilize heat-sensitive medical equipment such as the mechanical ventilator and incubators for neonates. Unfortunately formaldehyde is a mutagen and a potential human carcinogen, therefore must be regulated and fully contained to guarantee the permissible exposure limit of healthcare workers for formaldehyde.

7. Gaseous chlorine dioxide.

8. Vaporized peracetic acid.

9. Infrared radiation.

Steam Sterilization

- It is accomplished in an autoclave and uses moist heat in the form of saturated (dry) steam under pressure for a specified exposure time and at a specified temperature, as the sterilizing agent.
- Thus, there are four parameters of steam sterilization: steam, pressure, temperature, and time.
- The ideal steam for sterilization is saturated steam. It is essential to make steam saturated which means free of air because air acts as an insulator and hinders penetration.



- Pressure serves as a means to obtain the high temperatures necessary to quickly kill microorganisms.
- The two common steam-sterilizing temperatures are 121°C and 132°C . These temperatures must be maintained for a minimal exposure time to kill microorganisms. Recognized minimum exposure periods for sterilization of culture media or healthcare supplies are 30 minutes at 121°C in a gravity displacement autoclave or 4 minutes at 132°C – 135°C in a prevacuum autoclave.
- As regard mode of action, moist heat destroys microorganisms by coagulation and denaturation of enzymes and structural proteins.
- The two basic types of steam sterilizers are the gravity displacement autoclave and the prevacuum autoclave. In the former, incoming steam displaces residual air through a drain in the bottom of the sterilizer chamber. Typical operating temperature is 121°C . The gravity displacement autoclaves are used for culture media, water, and nonporous articles whose surfaces have direct steam contact.

What advantage?

The prevacuum sterilizers are similar to the gravity displacement sterilizers except they are fitted with a vacuum pump to ensure air removal from the sterilizing chamber and load before the steam is admitted. The advantage of using a vacuum pump is that there is instantaneous steam penetration. This method results in shorter cycle times because of the rapid removal of air and the usually higher temperature (132 – 135°C).

- Steam sterilization is nontoxic, inexpensive and rapidly heats and penetrates fabrics. It is the most widely used and the most reliable.

• **Monitoring of steam sterilizers (autoclaves)** use following 3 monitors:

1. **Mechanical indicators:** using a printout or graph that monitors the time, temperature and pressure of the sterilization cycle.
2. **Chemical indicators or integrators:** chemically impregnated paper strips that must be used with each sterilization cycle to monitor the temperature or time and temperature. Visible colour changes occur at specified temperature and time.
3. **Biological indicators:** paper strips impregnated with the spores of *G. stearothermophilus*. The biological indicators are placed at the coldest point of the chamber. After finishing the cycle of sterilization, spore strips are incubated in a fluid medium at 37°C for 48h. Absence of bacterial growth indicates an efficient sterilization cycle.

*How to test for efficiency of Autoclav.?

③ Mechanical indicator

↓ Chemical Indicators (Integrator)

↓ Biological indicators

e.g. $\Rightarrow$ *G. stearothermophilus* spores

Cup to
Not in oven
& autoclave

Infection
= organism + host relationship

Chapter 10

BACTERIAL PATHOGENESIS

Saprophytic
on decaying

Parasitic
on living

Infection is a process by which the organism enters into a relationship with the host. Although microbial infections occur frequently, most infections end without occurrence of pathological changes and thus are not manifested as clinical **disease**. These infections are termed **subclinical**, **silent** or **abortive** infections.

The outcome of bacterial infections depends on the mutual relationship between bacteria and host. Accordingly, bacteria could be classified into:

1. **Saprophytic bacteria**: are those which live freely in nature, on decaying organic matter, in soil or water. They do not require a living host.
2. **Parasitic bacteria**: are those which live on or in a living host. They are classified according to their relation to the host into:
 - a- **Pathogenic**: Bacteria capable of causing disease.
 - b- **Non-pathogenic (commensals)**: Bacteria that do not cause disease, and are part of the normal flora.
 - c- **Opportunistic pathogens**: These are potentially pathogenic bacteria that do not cause disease under normal conditions but can cause disease in immunocompromised patients, or when they find their way to another site other than their normal habitat. Many of these opportunistic pathogens are originally commensals.

Stages of the Infectious Process

1. **Source of infection** which may be man (case or carrier), animal or soil.
2. **Mode of transmission** e.g. droplet inhalation, ingestion, injection, insects, contact and transplacental.
3. **Portal of entry** e.g. respiratory tract, gastrointestinal tract, skin...etc. The organism then starts to multiply within the host causing tissue damage (disease).
4. **Portal of exit** e.g. urine, stools, blood, respiratory or genital discharge, from which the organism is transmitted to a new host.

Koch's postulates

1882 → مع احمد عيسى

These are criteria that were proposed by Koch in order to determine if the organism isolated from the patient actually caused the disease, i.e. these criteria must be satisfied to confirm the causal role of an organism. These criteria are as follows:

1. The organism must be isolated from every patient with the disease.
2. The organism must be isolated free from all other organisms and grown in pure culture in vitro.
3. The pure organism must cause the disease in a healthy, susceptible animal.
4. The organism must be recovered from the inoculated animal.

The outcome of infection depends on the interaction between microbial factors (virulence) and host resistance factors (immunity).



Microbial Virulence

While pathogenicity is a qualitative description of a species of bacteria denoting ability to produce disease, virulence is a quantitative character (degree of pathogenicity) of a strain belonging to a pathogenic species. Virulence is genetically determined by genes carried on plasmids, phages, pathogenicity islands and chromosomes.

Virulence Factors of Bacteria *structure or product enable organism to produce disease*
A virulence factor is either a structure (e.g. capsule) or a product (e.g. toxins) that enables the organism to cause disease.

A- Adherence factors *fimbriae, glycoCalyx*

They enable bacteria to attach to the host surfaces, thus contributing to the establishment of the infection. For example:

1. The fimbriae of *Neisseria gonorrhoeae* and *E. coli* help the attachment of these organisms to the urinary tract epithelium.
2. The glycoCalyx of *Staphylococcus epidermidis* and certain viridans streptococci allows the organisms to adhere strongly to the heart valves.

Mutants that lack these factors are often avirulent.

B- Invasion factors *enzyme, Antiphagocytic factors, Toxin production*

Invasion of tissue followed by inflammation is one of the main mechanisms by which bacteria can cause disease. This invasion is helped by:

1. Enzymes

- a- Collagenase and hyaluronidase which degrade collagen and hyaluronic acid and allow the bacteria to spread through subcutaneous tissues.
- b- Immunoglobulin A protease which degrades IgA.
- c- Leukocidin which can destroy both polymorphonuclear leucocytes and macrophages.
- d- Deoxyribonuclease that breaks down DNA.
- e- Lecithinase that breaks down lecithin of cell membrane.

2. Antiphagocytic factors

- a- Capsule: The capsule prevents the phagocytes from attachment to the bacteria, e.g. *Strept. pneumoniae*.
- b- Cell wall proteins of Gram-positive cocci, such as the M protein of *Strept. pyogenes* and protein A of *Staph. aureus*.
- c- Coagulase: It accelerates the formation of a fibrin clot from fibrinogen. This clot can protect bacteria from phagocytosis, e.g. *Staph. aureus*.

C- Toxin production

Toxin production is another mechanism by which bacteria can produce disease. Bacterial toxins are either exotoxins or endotoxins (Table 4).

Pathogenicity

Ability to produce disease
Qualitative

Virulence

degree of pathogenicity
Quantitative

Table (4): Comparison of the main features of exotoxins and endotoxins:

	^{+ve} Exotoxins	Endotoxins ^{G^{-ve}}
Source	Secreted by living organisms both Gram-positive (mainly) and Gram-negative ^{not only} = diffusible ptn	Integral part of the cell wall of Gram-negative organisms. Liberated upon cell disintegration
Coding genes	Encoded by chromosomes, plasmids, bacteriophages or PAI	Encoded by genes on the <u>chromosome</u>
Examples	<i>C. diphtheriae</i> (phage) <i>Cl. tetani</i> (plasmid) <i>B. pertussis</i> (chromosome) <i>H. pylori</i> (PAI) <i>Can be detoxified</i>	<i>E. coli</i> and meningococcal endotoxins
Nature	Protein	Lipopolysaccharide (lipid A) [↑]
Antigenicity	Highly antigenic ^{bec.}	Poorly antigenic ^{bec.}
Heat stability	Unstable to temp. above 60°C = Labile	Stable to temp. above 60°C for several hours ^{***}
Detoxification	Can be converted into <u>toxoid</u> *	Can not XXX
Specificity	Every toxin has specific action	Same generalized effect (non-specific action), all give fever and shock = endotoxic shock
Toxicity	High	Low

* Treatment of exotoxin with formalin (or other agents) removes its toxicity and retains its antigenicity converting it into toxoid, that can be used for immunization.

IMMUNOLOGY

د. مرعی ← Ch 12.

OVERVIEW OF THE IMMUNE SYSTEM

Immunology is a relatively new science which has always been linked to the science of microbiology. This is because the main function of the immune system is to combat infections caused by different microbes.

The immune response to infections includes two parts:

I. Innate Immunity *not for diagnosis = non specific*

This is present in all normal individuals since birth and at all times. It includes many different resistance mechanisms such as barriers, chemicals and cells present in the body. It is very important at the beginning of an infection. However, it is not always successful in eliminating infectious organisms on its own. It does not increase with repeated exposure and cannot tell the difference between different pathogens.

II. Acquired Immunity (Adaptive immunity) = specific

This is a specific immune response directed against a particular pathogen. It occurs during the lifetime of a person as an adaptive response to infection with that particular pathogen. It is associated with the development of immunological memory, so that in many cases it gives life-long protection against that same pathogen. It is very effective in combating infection, but there is a delay of a few days until it can start being effective.

Comparison between innate and acquired immunity is presented in table 5 (Chapter 12). *49.P*

The Components of the Immune System

The granulocytes, monocytes and lymphocytes are the main cells of the immune system. Innate immunity depends mainly on the granulocytes (neutrophils, eosinophils and basophils) and monocytes, while acquired immunity depends mainly on the lymphocytes. However, there is a great inter-connection between the cells and functions of the innate and acquired immune systems, as will be seen later.

Following is a brief description of the role of each cell in the immune response:

- Neutrophils** are phagocytic cells that are capable of engulfing and digesting foreign agents. They are the most numerous and most important cells of the innate immune system. Although they are short-lived (few hours), yet they form 60-80% of total blood leucocytes. They are absent from normal tissue, but when there is an infection at a certain place, certain chemicals (chemotactic factors) are released to attract neutrophils from the blood to the site of infection. Pus is formed mainly of dead neutrophils.

- Eosinophils** are mainly of importance in defence against helminthic parasite infections. They may kill such parasites by releasing the toxic contents of their granules onto such extracellular parasites. They also play an important role in allergic reactions, which are reactions that occur in some people against harmless antigens present in the environment. Eosinophils also have phagocytic properties.

Give function
Eosinophils = allergy or parasitic

Innate → granulo
Acquired → Lymphocyte

Innate
Acquired (adaptive)

قراءة

Reading

قراءة

قراءة

P.49

granulocytes
Monocyte + macrophage
Lymphocyte

granulocyte
Neutro eosino baso

in blood
also in blood
Submucosa
monocytes in blood
Macros in tissue

Basophils are found in the blood in very low concentrations. Their function is probably similar to that of mast cells.

Mast cells are found in the tissues either around the blood vessels or in the submucosa. They play a very important role in allergic reactions. They possess granules containing a number of important mediators such as histamine. Release of these mediators leads to the manifestations of allergy and inflammation.

Monocytes and Macrophages: Monocytes are present in the blood and continuously leave it to go to the tissues where they complete their maturation and become macrophages. Examples are the Kupffer cells of the liver and the alveolar macrophages in the lung.

Functions of Macrophages *enumerate + mention function*

1. **Phagocytosis** (Chapter 12).
2. **Antigen presentation:** Macrophages help to 'show' or 'present' part of the foreign agents they have eaten to T cells, so that the T cells can start responding to them. Thus, they are among a group of cells called **antigen presenting cells (APCs)**.
3. **Secretion:** They secrete chemical mediators called **cytokines**, e.g. interleukins.
4. **Direct cytotoxicity:** They may kill targets without engulfing them. Helminthic parasites which are too large to be engulfed can be killed by macrophages releasing their toxic contents onto them. Tumour cells can also be killed in a similar way.

Lymphocytes: include T and B lymphocytes as well as natural killer cells:

- **T lymphocytes** are produced in the bone marrow, but complete their maturation in the thymus. They comprise around 75% of peripheral blood lymphocytes. *glands when thymus atrophies in adult mature in thymic like hormone of Liver or hypothalamus*

- There are two main kinds of T cells:
1. **T helper cells**, whose main function is to secrete cytokines which help other cells of the immune system. *CD4 marker*
 2. **T cytotoxic cells**, whose main function is to kill infected cells and other abnormal cells, such as tumour cells. *CD8 marker*
- The ratio of T helper/T cytotoxic cells is around 2:1.

- **B lymphocytes** are produced in the bone marrow, where they complete their maturation. They comprise around 10% of peripheral blood lymphocytes. When B cells become active, they change into plasma cells which secrete protein molecules called **antibodies**, or **immunoglobulins**. These antibodies then circulate in the blood and have a very important role in many immune reactions.

Natural Killer cells (NK cells) are large granular lymphocytes which can be distinguished from B and T lymphocytes. They constitute 10-15% of peripheral blood lymphocytes. They are capable of killing abnormal or infected cells in a manner similar to cytotoxic T cells, but differ from them in the way they recognize their targets.

3 functions
tumour killer



The Lymphoid Organs

They are defined as organized tissues where lymphocytes interact with other non-lymphoid cells that are important either in their maturation or in starting an acquired immune response. They are divided into:

I. Primary (central) lymphoid organs

This is where lymphocytes complete their maturation, becoming **mature** (adult) lymphocytes. They are:

- **The bone marrow:** where the B cells complete their maturation. (Thus, the bone marrow is not only the place of origin of all blood cells, but also acts as a central lymphoid organ).
- **The thymus:** where the T cells complete their maturation.

II. Secondary (peripheral) lymphoid organs

They are the places where lymphocytes can meet **antigens**, leading to activation of the lymphocytes. The secondary lymphoid organs include the spleen, lymph nodes and various **mucosal associated lymphoid tissue (MALT)**.

- **The lymph nodes** are highly organized structures. The B lymphocytes are localized in follicles in the cortex of the lymph node, while T lymphocytes are more diffusely distributed in the paracortical area.

- **The spleen** is mainly composed of red pulp, which is the site of RBC disposal. The lymphocytes surround the arterioles entering the organ, forming the white pulp. The inner part of this is called the periarteriolar lymphoid sheath, containing mainly T cells and is surrounded by a B cell corona.

The gut-associated lymphoid tissue (**GALT**) includes the tonsils, adenoids, appendix and Peyer's patches.

The bronchial-associated lymphoid tissue (**BALT**) includes similar but more diffusely organized collections of lymphocytes which protect the respiratory epithelium.

- Other mucosal sites contain similar collections.

B	T
Bone marrow	Thymus
Lymph Cortex	Lymph Para Cortic
spleen Corona	spleen periart
Whit pulp	Whit pulp



Circulation of Lymphocytes between Blood and Lymph: (Fig. 11)

- 1- Small B and T lymphocytes that have matured, but have not yet met antigen are called naïve lymphocytes. They leave the bone marrow and thymus, respectively, and circulate continually from the blood into secondary lymphoid organs, such as the lymph nodes.
- 2- Microbial antigens are drained from the site of infection through the afferent lymphatic vessels into the lymph nodes. Not any lymphocyte seeing an antigen will recognize it. This is because lymphocytes are very specific for the antigens they recognize. Lymphocytes which recognize a certain antigen undergo a series of changes which make them ready to start working against the antigen to which they are specific.

The changes which occur are:

- a. Activation:** they become lymphoblasts.
- b. Proliferation:** rapid multiplication. = replicate
- c. Differentiation:** they change into **effector** cells, capable of being effective against the invading agent:

* B → plasma → antibody
* T → Helper → Cytokines

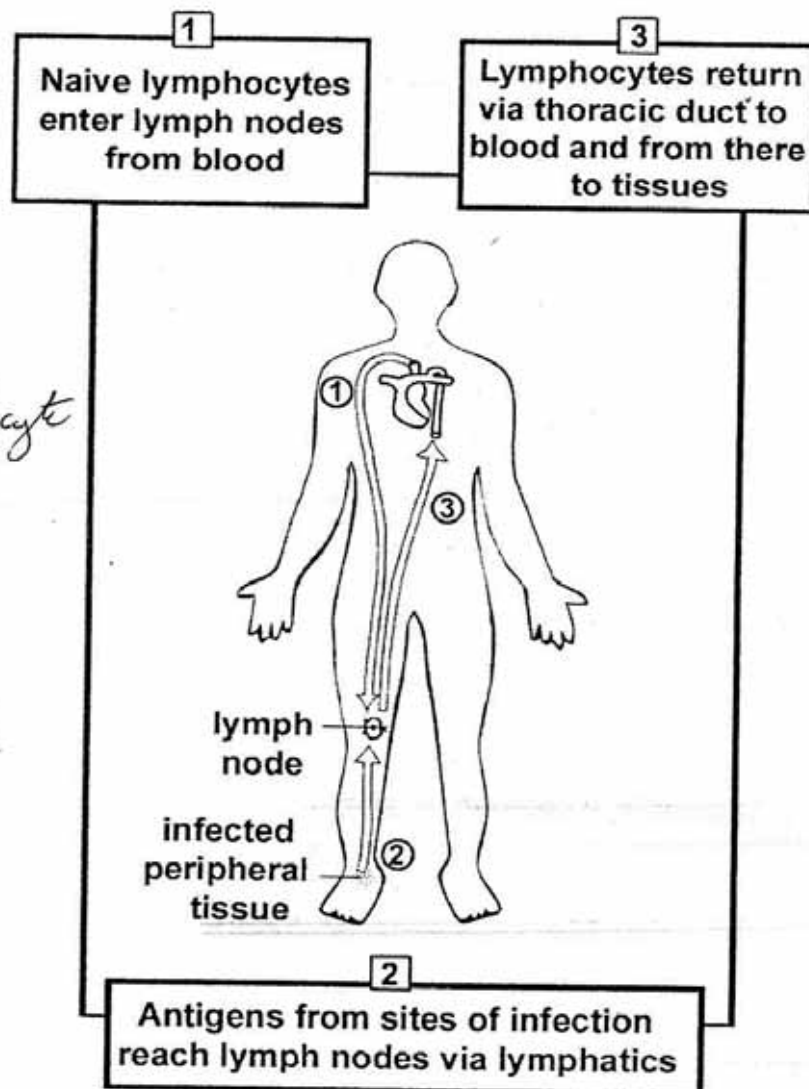
What changes occur to lymphocyte when recognize antigen?

White lymphoblasts



Diff. also!
differentiation

- The B cell changes into a **plasma cell**, capable of secreting antibody.
- The cytotoxic T cell becomes capable of killing infected cells.
- The helper T cell becomes capable of producing cytokines.



* Naive lymphocyte
of T cell
Antigen
or Sol
↓
Lymph node

Fig. (11): Circulation of lymphocytes between blood and lymph.

3- They now leave the lymph nodes through the efferent lymphatic vessels and return to the blood through the thoracic duct. From the blood, they reach the peripheral tissues where they start functioning to eliminate the specific infection which started their activation.

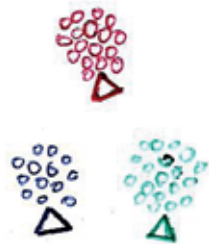
Some of the antigen-specific lymphocytes produced by these events remain after the antigen has been eliminated. These are called **memory cells** and are the basis of **immunological memory** which ensures a more rapid and effective response on a second meeting with the same pathogen and, therefore, gives long lasting immunity. Immunological memory is the most important biological sequence of the development of acquired immunity.

G.R. Why more rapid & efficient response in 2nd Time?
↓
memory cells formed due to 1st = Cohesive cells → more rapid
42



The Clonal Selection Theory

As mentioned above, T and B lymphocytes are very specific. They recognize antigen by receptors present on their surface. Every naive lymphocyte has a single type of receptor of a certain specificity. Only those lymphocytes which meet the antigen which their receptors recognize, will undergo activation, proliferation and differentiation. The result is a clone (family) of identical daughter cells, all having identical receptors, which can bind the same antigen wherever they find it. Thus, antigen specificity is maintained in the daughter cells. This is called the clonal selection theory (so-called because a certain **clone** of cells is **selected** from the pool of naive lymphocytes).



Lymphocytes having receptors specific for self molecules (a person's own molecules) are normally deleted at an early stage in lymphoid cell development and are, therefore, absent from the pool of mature lymphocytes.

All the above applies to both T and B lymphocytes. However, there are certain **differences between T and B cell receptors:**

1. The B cell receptor is actually an immunoglobulin which has two antigen recognition sites, while the T cell receptor has only one recognition site.
2. B cell receptors can be secreted (as antibodies in the blood and body fluids), while the T cell receptor is always a cell-surface molecule.
3. The B and T cell receptors recognize antigen in different ways:
 - The B cell receptor can recognize an antigen directly.
 - The T cell receptor cannot recognize antigen directly. Another cell, called the **antigen presenting cell (APC)** must take up the antigen inside it, break it up into peptides, then 'present' or show these peptides to the T cell receptor.

Recognition & Effector Mechanisms of Acquired Immunity

These are the mechanisms by which pathogens are detected and destroyed in a successful acquired immune response. It is very important to know that different pathogens have different lifestyles and, therefore, need different mechanisms for detection, recognition and destruction:

- B cells recognize antigen outside cells, where most bacteria are found.
- T cells can detect antigens generated inside the cell, as those belonging to viruses or intracellular bacteria.

I. Effector Mechanisms of Antibodies

Antibodies work mainly to eliminate extracellular pathogens and their toxins. Antibodies are found in plasma and extracellular fluids. Immunity mediated by antibodies is called **humoral immunity**. It is so-called because body fluids were once known as **humors**.

In general, when antibodies bind to pathogens, they help in combating infection in many ways. They may block access of the pathogen to body cells. They may also start a process which eventually leads to lysis of the pathogen. All pathogens bound by antibody are eventually delivered to phagocytes for ingestion, degradation and removal from the body.

الامتحان من ١٩

تسليم الامتحانات
١٩

II. Effector Mechanisms of T Cells

Pathogens are accessible to antibodies only in the blood and extracellular spaces. However, all viruses and some bacteria and parasites replicate inside cells, where they cannot be detected by antibodies. The destruction of these invaders is the function of the T lymphocytes. This is called **cell-mediated immunity**. In addition, T cells offer important help to B cells. The functions of T cells may be summarized as follows:

1. Cytotoxic T (Tc) cells

These recognize body cells infected with virus. Antigens from replicating viruses are displayed on the surface of infected cells, where they are recognized by the cytotoxic T cells. These cells directly kill the infected cells before viral replication is complete and new viruses are released to infect other cells.

2. Helper T (Th) cells

The function of these cells is mainly production of cytokines. There are two main kinds of helper T cells, grouped according to the type of cytokines they produce and the main cells they help:

a- T helper 1 (Th1) cells

These cells mainly secrete cytokines which help in **activation of macrophages**. This means making macrophages more capable of killing any bacteria inside them. This is very important because some bacteria, such as *M.tuberculosis*, after being ingested by macrophages, resist digestion and can survive for a long time inside.

b- T helper 2 (Th2) cells

These cells play a very important role in destruction of extracellular pathogens. They secrete certain cytokines which help in **activation of B cells**, so that they can become plasma cells and produce antibodies to deal with those pathogens.

It is important to remember that the decision whether Tc cells or Th cells are activated is not something which occurs by chance, but is directly dictated by the type of pathogen and its lifestyle. The result is that the type of immune response which occurs is exactly suitable for combating that particular infection. How this happens will be explained in the chapter on T cell-mediated immunity (Chapter 14).

Failure of Host Defence Mechanisms

In some cases, a certain aspect or aspects of the immune system are deficient and the immune system is unable to free our body of infections. This is known as **immunodeficiency**. In very severe immunodeficiency diseases, adaptive immunity is completely eliminated and death occurs early in life. In less severe failures, there are specific recurrent infections. AIDS is a disease in which a virus infects T helper cells and destroys them. The person suffers from infections in which T helper cells normally play an important role in defence.

→ not harmful immune response



Harmful Immune Responses

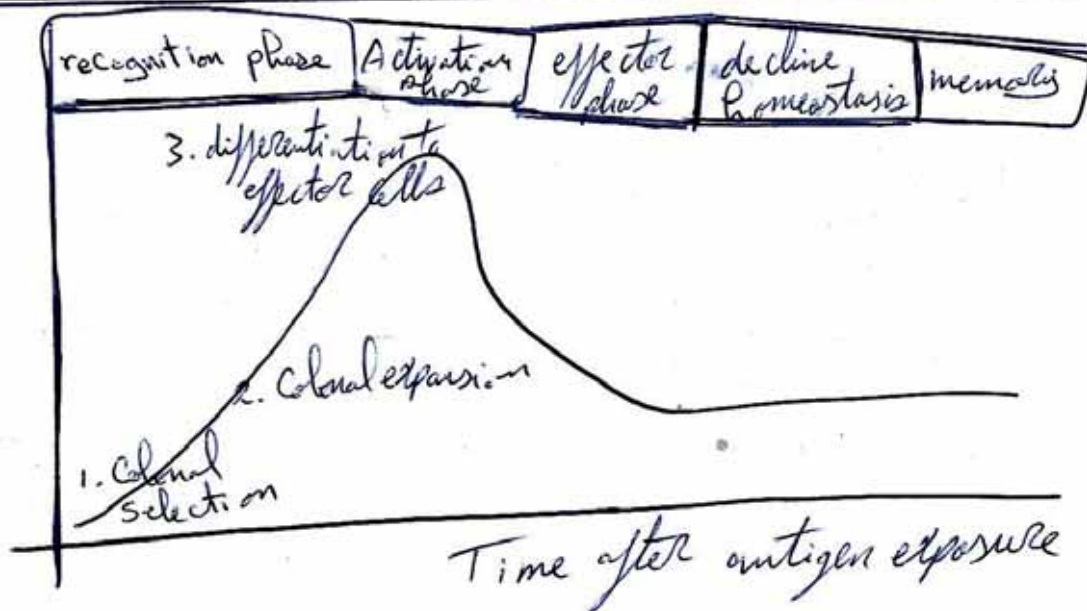
The work of the immune system is usually of benefit to the body, as in elimination of infections or in dealing with tumour cells. On the other hand, some immune responses may be harmful. Many medically important diseases are associated with such immune responses:

- **Allergy:** Inappropriate immune response against certain antigens.
- **Autoimmune diseases:** immune response against the person's own antigens.
- **Graft rejection:** immune response against foreign tissues or organs transplanted in a person.

In all the above conditions, the immune response causes harm, not benefit. Thus, whether we consider an immune response harmful or beneficial, depends not only on the response itself, but on the nature of the antigen.

Manipulation of the Immune System

- At present, the usual way to treat unwanted immune responses (allergy, autoimmune diseases and graft rejection) is by immunosuppressant drugs, which inhibit all immune responses, destructive and undestructive. If, instead, it were possible to suppress only those lymphocytes responsible for unwanted (bad) responses, the disease would be cured without affecting protective (good) immune responses. This dream of **antigen-specific immunosuppression** is the subject of much research but has not been very successful till now.
- On the other hand, **antigen-specific immunostimulation** (stimulating the immune system against a particular antigen or group of antigens) has been more successful. This is done through vaccination, which involves introduction of pathogens or parts of them, in a harmless form, into the body, in order to stimulate the immune system to produce an immune response to that particular pathogen. The result is that when the person is later exposed to the same pathogen in its harmful form, he/she is ready to combat that infection because of immunological memory.



Chapter 12

Immune system

Acquired

INNATE IMMUNITY

Non specific

Any individual is protected from potentially harmful microorganisms in the environment by a number of very effective mechanisms present since birth. They do not depend upon prior exposure to any particular microorganism. They are non-specific and can act against any microorganism or foreign invader.

Mechanisms of Innate Immunity

I. Mechanical barriers and surface secretions

1. Intact skin and mucous membranes constitute a barrier that cannot be penetrated by most microorganisms.
2. The sticky mucus covering mucous membranes traps any foreign material.
3. Cilia of the respiratory tract epithelium sweep foreign material out.
4. Blinking, sneezing and coughing reflexes expel foreign particles.
5. Sweat and sebaceous secretions contain substances (e.g. lactic acid and ammonia) that inhibit microorganisms.
6. Saliva, tears and mucous secretions of respiratory, alimentary and genitourinary tracts contain lysozyme which is bactericidal.
7. Gastric and vaginal acidity inhibit growth of microorganisms.
8. The flushing action of saliva, tears and urine helps in washing microbes from the body.

II. Normal bacterial flora

1. Bacteria of the normal flora produce bacteriocins and acids that destroy microorganisms.
2. They compete with pathogens for essential nutrients.

N.B.: Suppression of normal flora by antibiotics may lead to infection with potential pathogens (superinfection).

III. Humoral defence mechanisms

A number of microbicidal substances are present in the tissue and body fluids. They include:

1. **Lysozyme**: It is an enzyme that lyses bacteria by destroying the peptidoglycan of their cell wall.

2. **Complement**: It is a group of plasma proteins that act together to attack extracellular pathogens. The complement components are present in an inactive form, and can be activated either spontaneously by certain pathogens (where it is considered to be part of innate immunity) or by antibody binding to the pathogen (where it is considered to be part of acquired immunity) (Chapter 17).

3. **Acute phase proteins**: These are present at very low levels in normal serum, but their concentration rises dramatically shortly after the onset of an infection. Microbial products, e.g. endotoxins, stimulate macrophages to

Lysozyme

Mechanical - Present in saliva + tears + mucus
Humoral

microbe infection → endotoxin → macrophage → IL1 + IL6 →

release interleukin-1 (IL-1) and IL-6 which, in turn, stimulate the liver to produce a large number of acute phase proteins. They act to limit the spread of the infectious agent or stimulate the host response. Examples include fibrinogen and C-reactive protein (CRP) which helps in complement activation.

Acute phase
proteins
i.e.

fibrinogen
CRP
(Localized infection)
+
(activate Complement)

4. **Interferons:** There are 2 types of interferons. Type I Interferon (α and β) is part of innate immunity. It is secreted by virus-infected cells and prevents viral replication in uninfected neighbouring cells (i.e. it 'interferes' with viral replication).

N.B.: Type II (γ) Interferon is secreted by T cells and is part of acquired immunity.

IV. Cellular Defence Mechanisms

1. Phagocytes

Particles, e.g. bacteria, entering the tissue fluids or blood are rapidly engulfed by phagocytic cells. This process of engulfment (internalization) of particulate matter is termed phagocytosis. There are 2 main types of phagocytic cells, namely ① polymorphonuclear leucocytes (especially neutrophils) and ② mononuclear phagocytes (monocytes in the blood and macrophages in the tissues). Phagocytes contain digestive enzymes capable of degrading ingested material.

Innate non-specific
→ phagocytes
→ Natural killer
→ eosinophils

[No T no B
Acquired]

mononuclear
↓
In blood → monocyte
↓
in tissue → macrophage

→ Internalization
Phagocytosis (Fig. 12): The process of phagocytosis occurs in subsequent steps:

① Migration ② attachment ③ engulfment ④ killing

a- Migration (Chemotaxis): Microorganisms and injured tissues elaborate chemotactic factors that attract phagocytic cells to the site of infection. Some of the complement components and some cytokines may have chemotactic properties.

b- Attachment: Phagocytes have receptors on their surface that can recognize non-specific molecules common to many pathogens, allowing attachment to them. Some microorganisms may lack these molecules or cover themselves with a thick capsule that is not recognized by any phagocyte receptor. Attachment may still occur if these microorganisms become coated by molecules which the phagocytes can recognize. These may be an antibody, a complement component or other molecules (e.g. CRP). In this case, the process is called opsonization and the substance which helped phagocytosis is called an opsonin.

not recognize thick capsule bec. have no molecules

N.B.: Opsonization by antibody is an example how acquired immunity (antibodies) can help innate immunity (phagocytosis).

c- Engulfment: The cytoplasmic membrane of the phagocyte surrounds the organism and encloses it in a vacuole termed phagosome. Lysosomes, which are bags of enzymes, then fuse with the phagosome forming phagolysosomes in which the engulfed material is killed and digested.

Phagosome is vacuole formed by cytoplasmic membrane of phagocyte surround organism in

d- Killing: This occurs by 2 mechanisms:

- **O₂ dependent mechanism:** After engulfment, there is a respiratory burst consisting of a steep rise in O₂ consumption. This is accompanied by an increase in the activity of a number of enzymes and leads to the generation of various reactive oxygen intermediates, such as hydrogen peroxide and singlet oxygen, which are lethal to microorganisms.
- **O₂ independent mechanism:** Once ingested, most microorganisms are digested by lysosomal enzymes (lysozyme, elastase, hydrolase ... etc.).

★ Give account

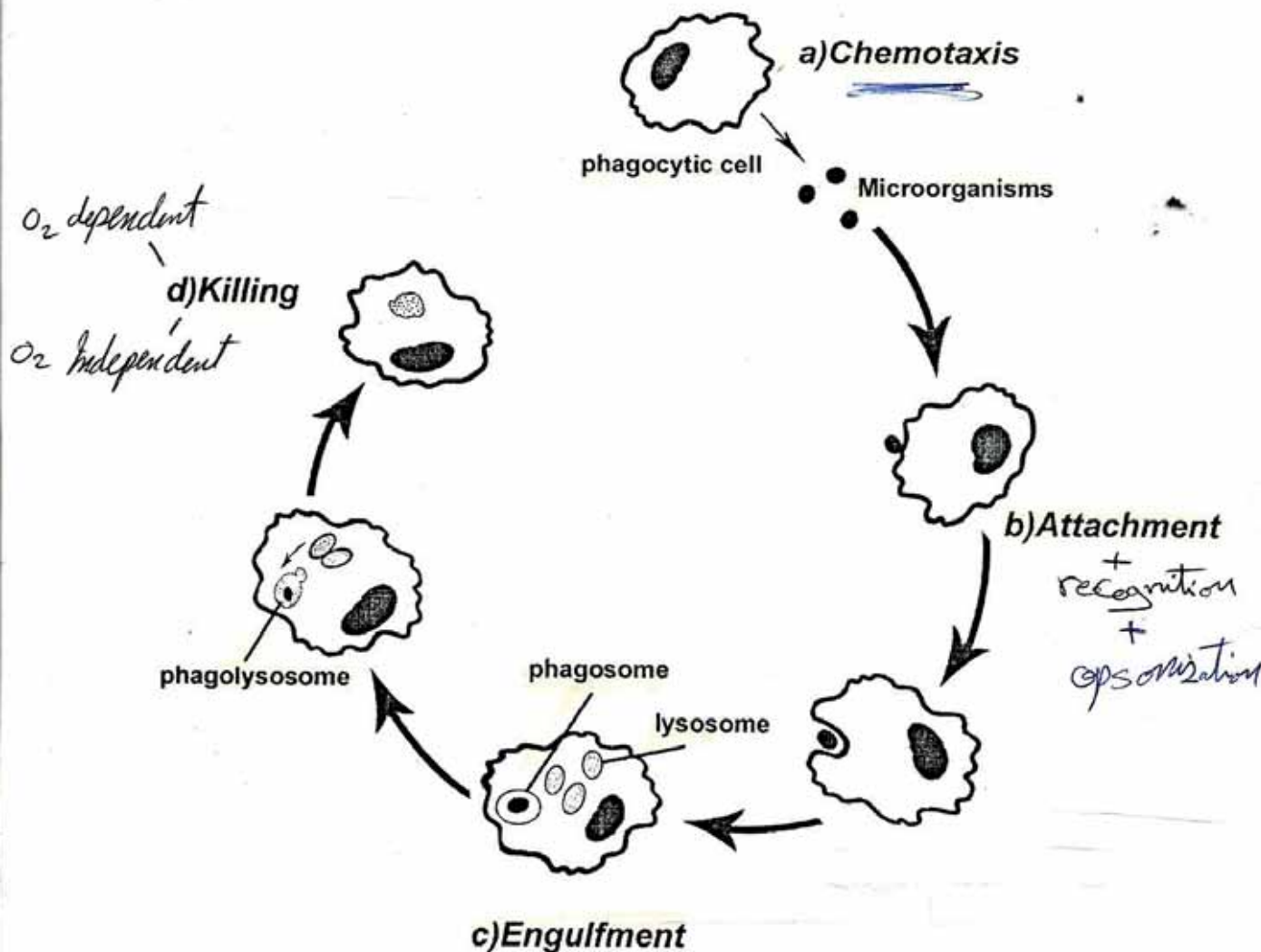


Fig. (12): Stages of phagocytosis.

2. Natural killer cells

These are large lymphocytes capable of causing non-specific killing of tumour cells and virus-infected cells by an extracellular killing mechanism. They comprise 10-15% of peripheral blood lymphocytes.

3. Eosinophils

Eosinophils have cytoplasmic granules that contain enzymes and toxic molecules that are active against parasites. The release of these molecules allows the extracellular killing of parasites that are too large to be phagocytosed, e.g. helminths.

V. Inflammation ^{Innate}

Chemical mediators released at the site of infection trigger an inflammatory response. The events that occur during inflammation are vasodilation, increased vascular permeability and migration of leucocytes from the blood stream across the vascular endothelium into the inflamed tissues to combat the invading microbe. This migration is mediated by certain molecules, termed adhesion molecules, which are expressed on the surface of leucocytes and vascular endothelium.

① Vascular permeability
② migration of leucocytes
③ by adhesion molecules

Host Factors Governing Innate Immunity

- Species factors:** Some organisms can produce disease only in certain species, e.g. *Mycobacterium leprae* can cause disease in man only.
- Racial factors:** Certain races are more susceptible to infection by some organisms, e.g. dark races are more susceptible to tuberculosis.
- Individual factors:** Some individuals are more susceptible to microbial disease; this may be related to:
 - Age: Very young and very old people are more susceptible to infection.
 - Hormonal factor: e.g. people receiving corticosteroids have lower resistance to infection.
 - Genetic make-up and nutritional status of an individual may also play a role in the resistance to infection.

Table (5): Comparison between innate and acquired immunity

	Innate	Acquired
Presence	Since birth	Following exposure to pathogens
Onset of action	Immediately after infection ✓	Relatively delayed X
Main cells	Granulocytes, monocytes/macrophages & NK cells ④	B & T lymphocytes ②
Memory	Absent X	Present ✓
Efficiency	Less efficient X	More efficient and improves with each exposure ✓
Specificity	Non-specific: X Present in all individuals, against all microorganisms without previous exposure	Specific: ✓ Occurs in a given person, against a particular pathogen when exposed
Interaction	Interact with acquired immunity through: e.g. - Antigen presentation ✓	Interact with innate immunity through: e.g. - Opsonization ✓

Immunogen → produce immune response
 Antigen → مادة تثير الاستجابة المناعية
 Chapter 13

ANTIGENS = Immunogen

An immunogen is a substance that can stimulate the immune system to produce an immune response (humoral and/or cell-mediated) and reacts specifically with the product of this response. The terms immunogen and antigen are used interchangeably.

Antigenic Determinants or Epitopes

The immune system does not recognize the antigen molecule as a whole but reacts to structurally limited parts of the molecule called epitopes. They are very small, composed of just four to five amino acids or monosaccharide residues. They determine the specificity of the antigen. The same antigen may possess different epitopes. Antigens that share one or more similar epitopes are known as cross-reactive (heterophil) antigens (Fig. 13).

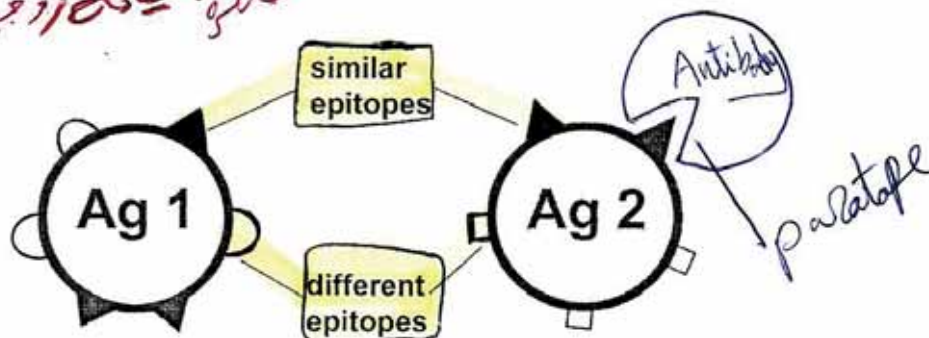


Fig. (13): Heterophil antigens.

Hapten

→ e.g. drugs
 Hapten + protein carrier → Antigen action
 This is a low molecular weight substance which is incapable of inducing immune response alone but when coupled with a carrier molecule (protein) it can act as an antigen. Examples of haptens are drugs e.g. penicillin.

Factors Affecting Immunogenicity

1. Foreignness

For a molecule to be antigenic, it must be foreign to the host in which it is introduced. The immune system can normally distinguish between body components (self) and foreign substances (non self) and is normally tolerant (non-reactive) to self antigens (autotolerance).

2. Molecular Size

Usually, the larger the molecule the stronger the antigenicity. However, there are exceptions; e.g. insulin is a small molecule but is immunogenic and carbon particles are very large but are non immunogenic.

* Give Reason: ^{the most is protein} $\uparrow$ Complex $\uparrow$ Antigenic $\uparrow$ immunogenic

3. Chemical Nature

The chemical complexity of a molecule contributes significantly to its immunogenicity. The more complex the molecule, the more immunogenic it is. The most potent immunogens are proteins.

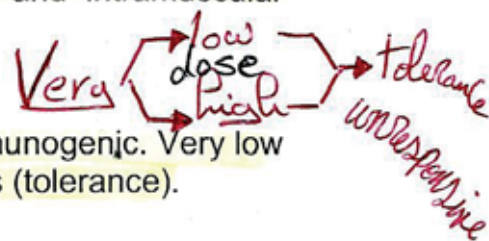
4. Route of Administration

No vaccine IV Give Reason

The route of administration of an antigen may affect the type and intensity of the immune response. As a general rule, the subcutaneous and intramuscular routes are the best in provoking an immune response.

5. Dosage

$\uparrow$ dose Antigen $\uparrow$ Antigenic



There is an optimum dose at which any antigen is most immunogenic. Very low or very high doses may result in a state of unresponsiveness (tolerance).

6. Adjuvants

Define

Adjuvants are non-specific potentiators of the immune response. The administration of an adjuvant together with an antigen enhances the immune response to that antigen.

One of the commonly used adjuvants is aluminium hydroxide which is added to diphtheria and tetanus toxoids that are used for human immunization. Aluminium hydroxide delays the absorption of toxoids and prolongs the period of their exposure to the immune system. $\rightarrow$ Adjuvant effect

7. Host Factors

The generation of an immune response is genetically controlled. Thus, some antigens stimulate an immune response in humans but not in animals. Also, the immune system may not respond to an antigen at the extremes of age (due to immaturity or aging of the immune system).

* Mention difference between Antigen & Hapten
* In chapter 15 P.15 $\Rightarrow$ Difference between Plasmid & Transposable element

Antigen

- ① Can stimulate immune response by itself
- ② Larger

Hapten

- ① Can't stimulate immune response by itself
- ② Small size + low M.W.
- ③ Need ptn to couple to act as antigen

Both have epitopes (Antigenic determinant)

naive = mature no experience with Antigen
*
not mature

Chapter 14

@destruct @help

T-CELL MEDIATED IMMUNITY

Acquired
Specific

The main functions of T cells are the destruction of intracellular pathogens as well as helping other cells of the immune system. For T cells to start functioning in the acquired immune response, they must first change from naïve T cells into effector T cells. Naïve T cells are activated to proliferate and differentiate into effector T cells the first time they recognize their specific antigen on the surface of an antigen presenting cell (APC). T cells recognize antigens by their receptors (T cell receptors) with the help of a number of other molecules present on the T cell. They can only recognize antigen when it is carried on special molecules called major histocompatibility (MHC) molecules, present on the surface of the APCs. Understanding T cell activation and function, requires understanding of the most important T cell surface molecules as well as the APCs and the MHC molecules.

T Cell Surface Molecules: (Fig. 14)

In addition to their role in antigen recognition and in interaction with other cells, these molecules serve as markers which help in identifying T cells and dividing them into subsets. The most important are:

1. **T Cell Receptor (TCR):** This receptor consists of two polypeptide chains called α and β chains. In a manner similar to antibody molecules, both chains of TCR have a constant part near the cell membrane and a peripheral variable region. According to the structure of the variable region, the T cell receptor can recognize a certain antigen. All TCRs on a single T cell are identical and recognize the same antigen.

PreCognition = TCR

② Interaction = CD28 $\rightarrow$ B7
CD40L (B & T)

③ T cell Markers = CD4 Helper
CD8 cytotoxic

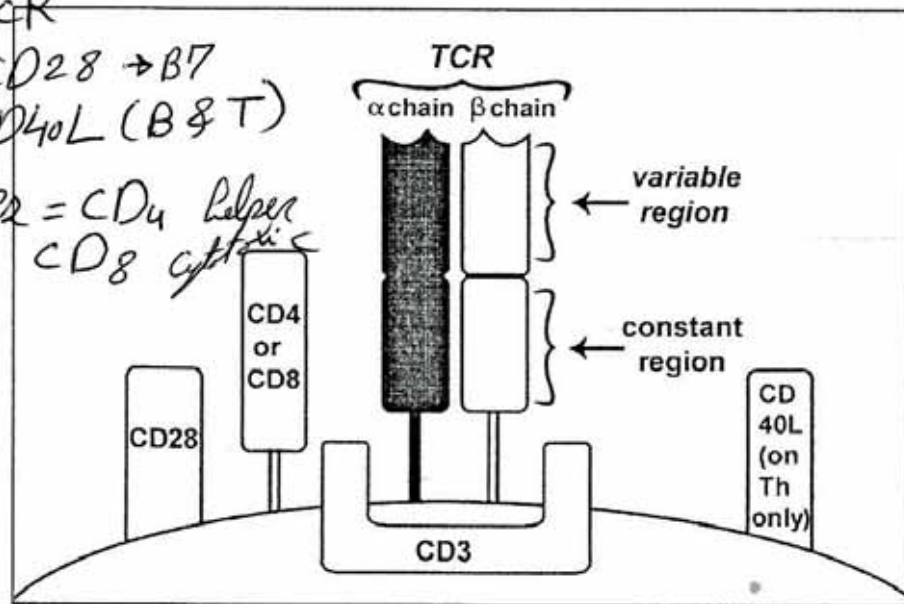


Fig. (14): T-cell surface molecules.

2. CD3 Molecule: This is found close to the TCR on all T cells and is involved in transmitting signals from the TCR to the inside of the cell.

3. CD4 and CD8 Molecules: T cells carry either CD4 or CD8 molecules on their surface and are thus classified into two major classes: *CD4 or CD8*

- CD4 T cells are called **helper T (Th) cells** and their main function is to **help** other cells of the immune system by secreting cytokines.
- CD8 T cells are called **cytotoxic T (Tc) cells** and their main function is to **kill** infected cells, tumour cells and other cells carrying on their surfaces antigens which the T cell recognizes.

CD4 and CD8 molecules are also associated with the TCR during antigen recognition and are, therefore, called **co-receptors**.

The signal delivered through the TCR, with the help of the above-mentioned molecules, is considered the **first signal** needed for T cell activation.

4. CD28 Molecule: This molecule is present on **all T cells**. During the process of antigen recognition, it binds to a molecule called B7 present on the **APC**. This binding delivers a **second signal** necessary for T cell activation. CD28 is, therefore, called a **co-stimulatory molecule**.

5. CD40 Ligand (CD40L): This molecule is present on activated T helper cells and is involved in activation of B cells by T cells. It binds to a molecule on B cells called CD40 (hence the name CD40L).

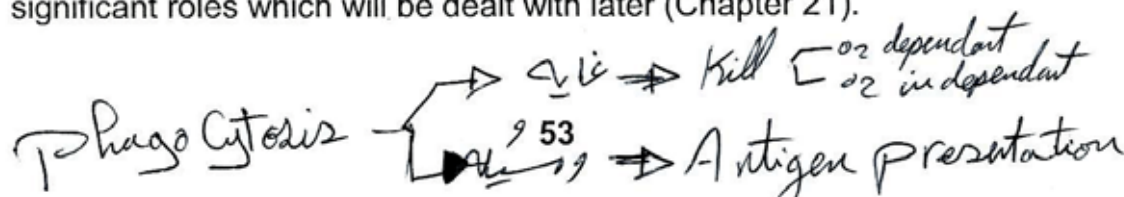
The Professional Antigen Presenting Cells

These are the only cells capable of **activating naïve T cells**. They are concentrated **in the peripheral lymphoid tissues**, such as **lymph nodes**, where they trap antigen and present it to the recirculating T cells:

- 1. Dendritic cells** are the most important **APCs**. They are so-called because they have cytoplasmic projections called dendrites. They are present in nearly all tissues of the body and are the most efficient APCs. Their only known function is **antigen presentation**.
- 2. Macrophages** are important phagocytic cells and are essentially cells of the innate immune system. Their action as APCs allows them to contribute to the acquired immune response.
- 3. B cells** are mainly known for their action in humoral immunity, but they can also act as APCs. *B cells immunoglobulin act as receptor*

MHC Molecules = (HLA) *Antigen presentation Transplantation [Ch 21]*

- The major histocompatibility complex (MHC) is the name given to a group of genes which code for the production of certain cell-surface **glycoproteins** called MHC molecules. Another name for them is the human leucocyte antigens (HLA antigens) because they were first discovered on the leucocytes. In addition to their role in antigen presentation, they have other significant roles which will be dealt with later (Chapter 21).





- There are two classes of MHC molecules which are important in T cell activation. They are called class I and class II MHC molecules (MHC I and MHC II). All people have both classes of MHC molecules.
- All nucleated cells of the body have MHC I molecules on their surfaces. The professional APCs have MHC II molecules, in addition to the MHC I molecules, on their surfaces.

Antigen Presentation to T Cells

e.g. leucocytes

For antigens to be presented to T cells, they must first enter inside the APC, undergo degradation (antigen processing) producing peptides which are carried inside clefts on the MHC molecules to the cell surface, to be presented to T cells (antigen presentation).

The decision of whether antigenic peptides will be presented by MHC I or MHC II molecules depends upon the part of the cell in which the pathogen is present. This will decide the type of T cell activated and the resulting immune response.

There are two compartments in our cells in which infectious agents or their products can exist: the cytosol and the vesicular system:

T Cytotoxic Killer

1. The Cytosol: This is the fluid cytoplasm. All viruses and few bacteria live and replicate in this compartment. Antigens from pathogens in this compartment are sometimes called 'endogenous' antigens.

T Helper

2. The Vesicular System: This includes the phagosomes, lysosomes and other intracellular vesicles:

- Some very important intracellular bacteria (such as that causing TB tuberculosis), as well as some parasites, are engulfed by macrophages, and can live and replicate in the phagosomes inside them.

agg + S = phagocytosed

- Other bacteria which normally live extracellularly secrete toxins and other proteins. Such bacteria or their products can also be internalized by cells, thus reaching the vesicular system of the cell. Some viral components are also secreted extracellularly and can be internalized into the vesicular system in a similar manner. B cells are very efficient at binding extracellular pathogens and their products by the antibodies on their surface and internalizing them. Dendritic cells are also capable of harbouring antigens in their vesicular system.

Antigens from pathogens in the vesicular system are sometimes called 'exogenous' antigens.

Important Rules:

- Peptides arising from the cytosol are carried to the surface of the cell on MHC I molecules and can only be presented to CD8 (cytotoxic) T cells.
- Peptides arising from the vesicular system are carried to the surface of the cell on MHC II molecules and can only be presented to CD4 (helper) T cells.



Handwritten signature or mark in the top right corner.

Table (6): Comparison between cytosolic and vesicular pathogens:

	Cytosolic "endogenous"	Vesicular "exogenous"
Examples	All viruses, few bacteria	-Intracellular bacteria -Extracellular bacteria and their products when internalized
Degraded in	Cytoplasm	Vesicles + vacuole + phagosome
Peptides bind to	MHC I molecules <i>all nucleated cells</i>	MHC II molecules <i>& MHC I APC only</i>
Presented to	CD8 T cells <i>Cytotoxic</i>	CD4 T cells <i>Helper</i>
Result	Cytotoxic killing of presenting cell by CD8 T cell	Secretion of cytokines by CD4 T cells, giving help to macrophages, B cells and others

موقوف على/خاصه

MHC Restriction = Recognition of Antigen by T Restricted by MHC
 This means that antigen recognition by T cells is restricted by the MHC molecules. This is true on two levels:

- Since CD8 T cells recognize peptides bound to MHC I molecules and CD4 T cells recognize peptides bound to MHC II molecules, it is said that **CD8 T cells are "MHC I restricted"** and **CD4 T cells are "MHC II restricted"**.
- There is a variety of different possible shapes of MHC I and MHC II molecules (**MHC polymorphism**). Thus, one cell may have many different MHC I and MHC II molecules, and there are even more differences between MHC molecules on cells of different people.

14 MHC *Carrying peptide dependent*

Not any MHC molecule can carry any peptide; this depends on the shape of the MHC molecule and the cleft inside it. The TCR is actually specific to the whole MHC-peptide complex, and not just the peptide alone. This means that a given T cell will recognize a certain peptide only when it is bound to a certain MHC molecule and will not recognize the same peptide bound to a different MHC molecule. Thus, recognition of antigen by T cells is restricted not only by the class of MHC molecule, but by the particular shape of the MHC molecule (Fig. 15).

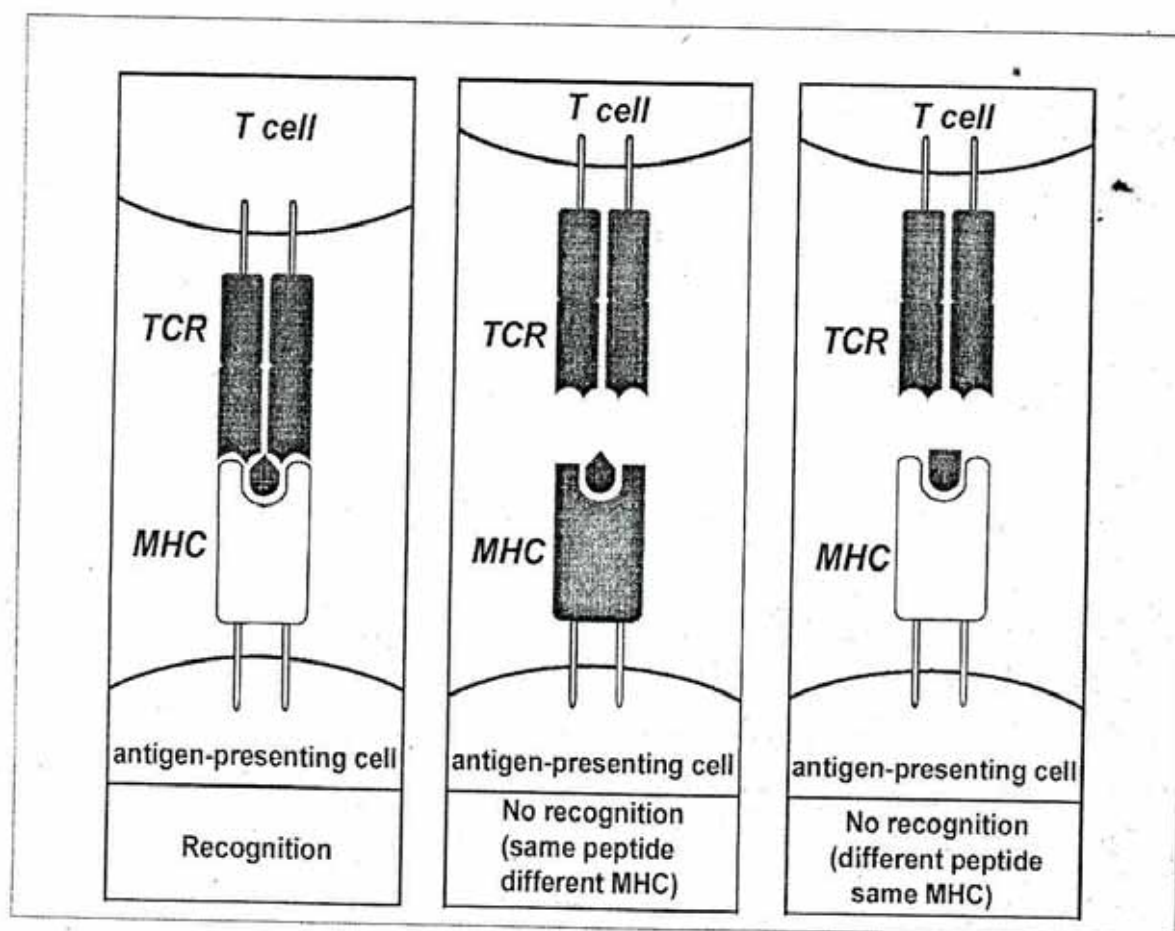


Fig. (15): MHC restriction.

Not if immature

Sequence of Events in Activation of Naïve T Cells: (Fig. 16)

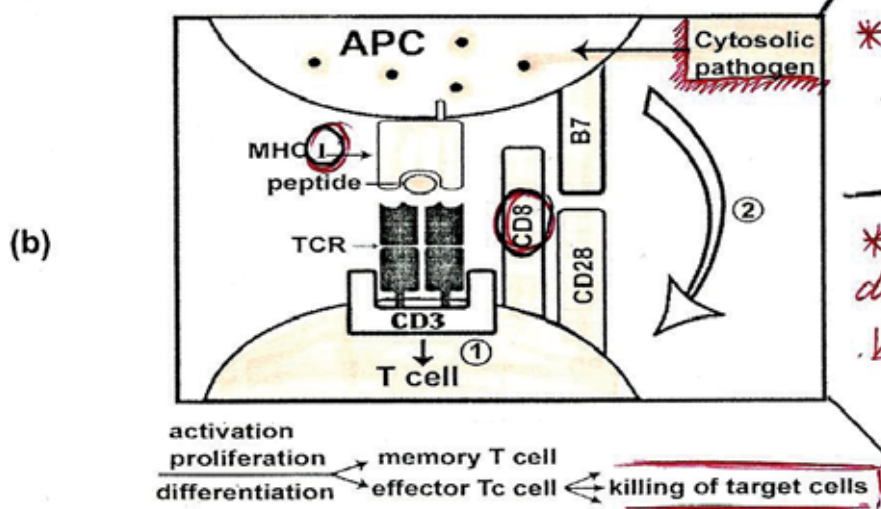
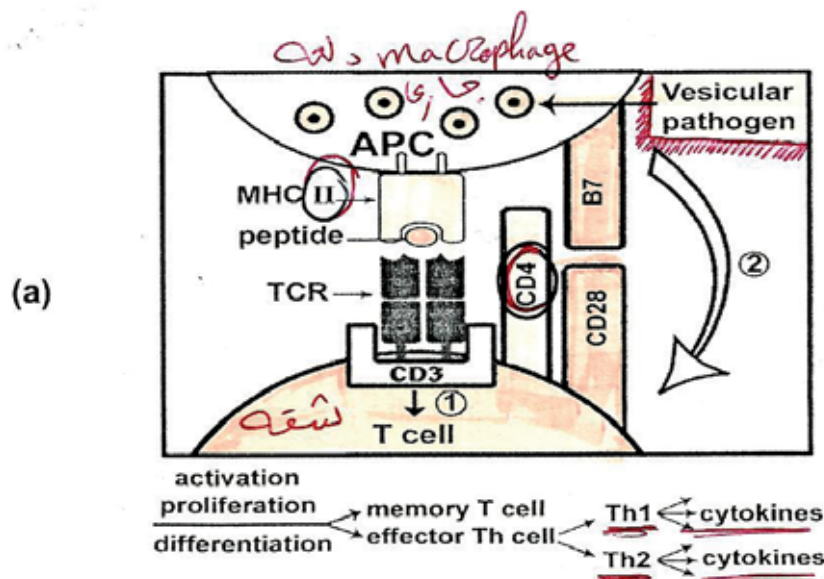
- Any cytosolic peptide is delivered on an MHC I molecule to the surface of the APC. A CD8 (cytotoxic) T cell with TCRs specific for that peptide binds the MHC I-peptide complex.
- Similarly, any vesicular peptide is delivered on an MHC II molecule to the surface of the APC. A CD4 (helper) T cell with receptors specific for that peptide binds the MHC II-peptide complex.
- In either case, this leads to delivery of the first signal required for T cell activation.
- The second signal is delivered by binding of CD28 on the T cell to B7 on the APC (co-stimulation). This signal is very important and without it the T cell 'shuts down' and becomes non-responsive, a state called anergy.

No response

2. That happens if MHC is not

- During these events, certain molecules, called **adhesion molecules**, present on the T cell and APC help to hold the two cells together.
- The T cell becomes a **lymphoblast** (activation), divides repeatedly (proliferation or clonal expansion) and its progeny (daughters) finally, become effector cells (differentiation). **Proliferation and differentiation** are helped by interleukin-2 (IL-2), a cytokine secreted by the T cell itself.

This sequence of events is known as the **primary immune response**. In addition to the production of **effector T cells**, it also results in the development of **memory T cells**, which are long-lived T cells that respond to antigen with an accelerated response and provide protection against subsequent challenge by the same pathogen (**secondary immune response**).



* CD3 on all T cells
near TCR
Transient signals
inside cell

* CD28 in all T cells
during Antigen recognition
bind B7 on APC
→ second signal
for T cell activation
= Co-stimulatory

Fig. (16): Activation of naïve T cells:
(a) Activation of Th cells, (b) Activation of Tc cells.

What is Importance of B7?
" " " " CD28?

* Differ between naïve and effector T Lymphocyte

• Effector cells differ from naïve T cells in many respects:

- They are able to respond quickly and efficiently when they encounter antigen on their target cells.
- They can be triggered to act as soon as their TCRs bind their specific MHC-peptide complex (signal 1), without need for co-stimulation (signal 2).

- The **effector cytotoxic T cells** are now capable of recognizing and killing any cell carrying on its surface MHC I-peptide complexes similar to the one which started the process of activation. The result is that any cell of the body infected with the same virus, for example, can be killed.

- The **effector helper T cells** start secreting cytokines. According to the type of cytokines produced, helper T cells are classified either as T helper 1 (Th1) cells which mainly activate macrophages or T helper 2 (Th2) cells which mainly activate B cells.

From the above it may be concluded that:

1. The lifestyle of the pathogen (where it lives and which compartment of the cell it enters) decides whether cytotoxic or helper T cells will be stimulated and consequently the type of immune response. The immune response in each case is exactly what is needed for defence against that pathogen:

- When the pathogen is in the cytosol (e.g. a virus), the only solution is to kill that cell by cytotoxic T cells since it harbours numerous virus particles which may infect other cells. This shows the importance of the MHC I → CD8 connection.
- When the pathogen is in the vesicular system, there is no indication for activation of cytotoxic cells and killing of cells. Vesicular pathogens, as mentioned previously, are either intracellular pathogens living inside macrophages, or are products of extracellular pathogens which have been internalized by B cells. In these cases, activation of helper T cells is needed. Cytokines released by helper T cells will help in activation of macrophages and B cells. This shows the importance of the MHC II → CD4 connection.

2. The significance of the presence of MHC I on all nucleated cells of the body is clear. Any cell of the body is liable to be infected by a virus, so all cells must possess MHC I molecules in order to be able to 'show' viral peptides to the Tc cell so that it can kill that infected cell; otherwise it would escape killing. In other words, the presence of MHC I molecules on all body cells is necessary so that these cells can be targets for the Tc cells if the need arises.

3. The significance of the presence of MHC II on only a few cells of the immune system (mainly the professional APCs) is also clear. Presentation of antigen to Th cells results in production of cytokines. These cause activation of many cells of the immune system, with many consequences. There is no need for the presence of MHC II on all cells of the body; in fact this would lead to over-stimulation of Th cells and over-production of cytokines, which would be dangerous to the body.

* Presentation to Naïve T
* Targeting to Cytotoxic
58

Lead to toxicity

سؤال امتحان دائم



بموقع الخلية لانها تفرز وتحتوي



Superantigens: (Fig. 17)

Certain proteins secreted by some pathogens do not act like ordinary antigens (Table 7). They are not processed and presented to T cells like ordinary antigens, but have the ability to bind directly to the MHC II molecule on the surface of the APC without entering the cell, and at the same time to the variable portion of the β chain of the TCR, acting as a clamp between both molecules. This type of binding to TCR is not very specific as in ordinary activation of T cells and, consequently, very large numbers of Th cells can be activated by one kind of superantigen. That is why these antigens are called 'superantigens'. The result is release of huge amounts of cytokines, which is not beneficial to the host and even causes systemic toxicity. There is suppression of the normal acquired immune response and no memory cells are produced.

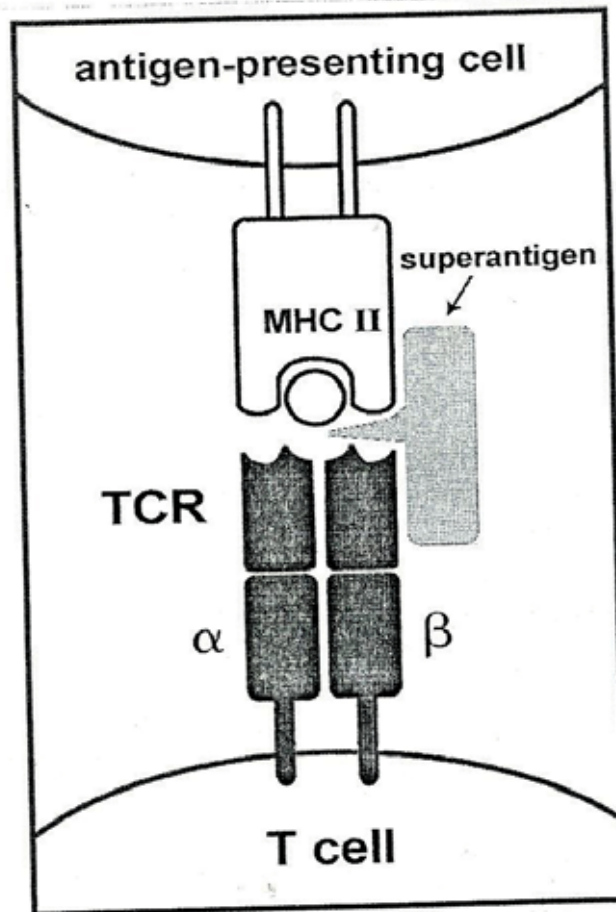


Fig. (17): Activation of T cells by superantigens.

Superantigens are produced by many different bacteria and viruses and are effective at very low concentrations. Well known examples of superantigens are staphylococcal enterotoxins and toxic shock syndrome-toxin. The overproduction of cytokines in response to these toxins accounts for many of their toxic effects on the body.



Table (7): Comparison between ordinary antigens and superantigens:

	Ordinary Antigen <i>specific</i> Yes	Superantigen <i>not specific</i> No
Processing inside APCs	Yes	No
Presentation by MHC molecules	Yes	No
Site of binding to MHC molecule	Peptide-binding cleft	Outside peptide-binding cleft
Binding to TCR	Variable portion of α and β chains	Variable portion of β chain
Specificity of TCR to it	Very specific	Not very specific
Acquired immune response	Stimulated	Suppressed
Development of memory	Yes ✓	No ✗
Result of T cell stimulation	Usually <u>beneficial</u> to host	Usually <u>harmful</u> to host
<i>e.g.</i> <i>dose</i>	H-Antigen + <i>somatic</i> <i>optimum nat. dose</i>	<i>enterotoxin</i> <i>Very low dose</i>

Functions of Effector T Cells

1. Function of effector CD4 T cells (Helper T cells): The main function of effector CD4 T cells is to secrete cytokines, and according to the panel of cytokines they secrete, they are classified either as Th1 or Th2 cells:

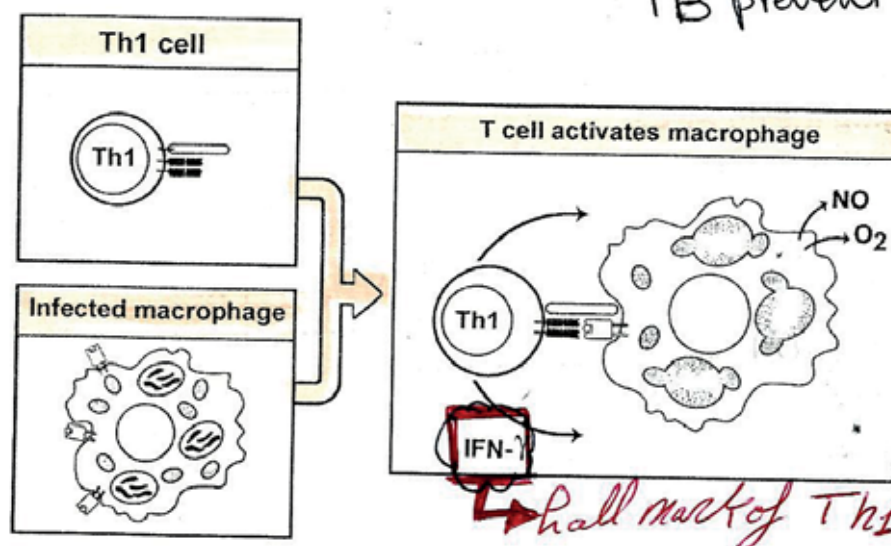
1. Th1 cells: produce cytokines which predominantly help macrophages and promote inflammation.

► Mechanism of activation of macrophages by Th1 cells: (Fig. 18)

- An infected macrophage displays a peptide-MHC II complex to an effector Th1 cell.
- If the Th1 cell is specific for that peptide-MHC II complex, it is induced to secrete cytokines, most important of which is Interferon- γ (IFN- γ). This causes activation of the macrophage, making it more capable of killing the bacteria it harbours:
 - There is more efficient fusion between the phagosomes containing the bacteria and the lysosomes containing the bactericidal enzymes.
 - There is also increased production of oxygen radicals, nitric oxide and antibacterial enzymes by the macrophage.

All this leads to effective killing of the intracellular bacteria.

Activation of Th1 cells and consequent macrophage activation can sometimes cause significant tissue damage. However, its absence can lead to serious consequences of disseminated infection. This is typically seen in AIDS patients with mycobacterial infection, since the number of Th cells in these patients is very low.



TB prevent phagocytosis formation

Fig. (18): Mechanism of activation of macrophages by Th1 cells.

2 Th2 cells: produce cytokines which predominantly help B cells and promote humoral immunity. Details of this are discussed in the chapter on humoral immunity (Chapter 16).

Details of the cytokines produced by Th1 and Th2 cells, and a comparison between both groups of cells are present after discussion of the various cytokines in Chapter 15.

II. Function of Effector CD8 T Cells (Cytotoxic T Cells)

The main function of effector CD8 T cells is to eliminate abnormal cells, such as virus-infected cells or tumour cells, which could be dangerous to the body as a whole.

- The target cell, such as a virus-infected cell, displays on its surface viral peptide in conjunction with an MHC I molecule.
- An effector Tc cell with TCR specific for that peptide-MHC complex recognizes it and delivers a lethal hit, leading to death of the target cell.
- After inducing death of the target cell, the Tc cell detaches and searches for other target cells to kill.

► Mechanism of killing by Tc cells: (Fig. 19)

Two mechanisms are involved:

1. **Induction of apoptosis:** The Tc cell induces the target cell to undergo suicide. This process of suicide is called **apoptosis**. It may occur by one of two mechanisms:

2 Mechanism
a- **Release of cytoplasmic granules:** The cytotoxic cell releases two kinds of granules called **perforins** and **granzymes**. The perforins create **perforations** (holes) in the **cytoplasmic membrane** of the **target cell**. Through these **holes**, **granzymes enter the target cell and cause activation of enzymes** naturally present in it, leading to degradation of the cell's DNA.

= Apoptosis



factor of apoptotic signal

b- Interaction of cell surface molecules: A receptor called **FAS**, present normally on many cells, binds to a molecule called **FAS-ligand**, present on cytotoxic cells, giving a signal for apoptosis in the target cell.

قتل نشین

Apoptosis is a clean death in which the cell destroys itself from within, shrinking and degrading itself until little is left. The enzymes which are activated to destroy cellular DNA can also degrade viral DNA, thus preventing release of viral particles to infect other cells.

Why Apoptosis is better against viral infection?

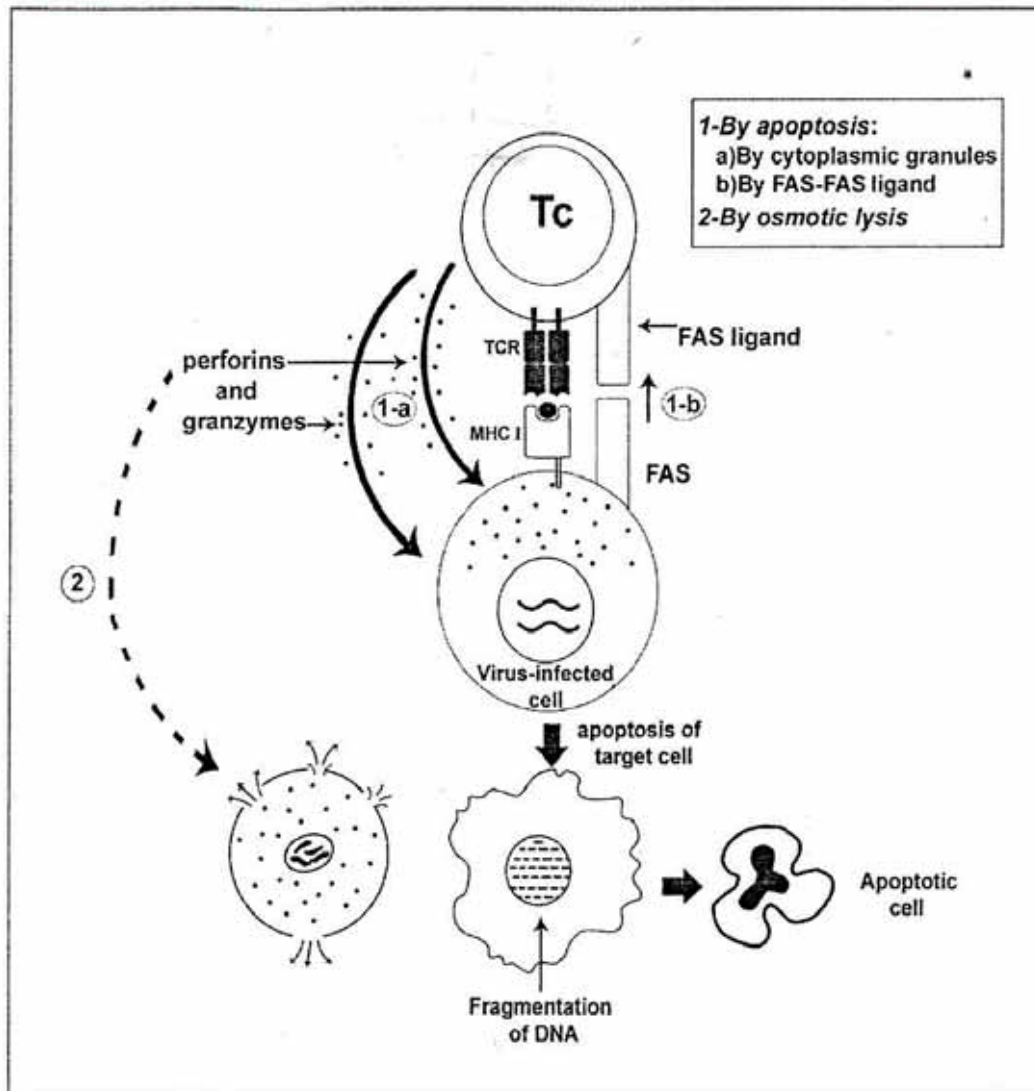


Fig. (19): Mechanism of killing by Tc cells.

2. Osmotic lysis: The pores produced in the target cell membrane by the perforins may allow entrance of fluid into the cell, leading to death of the target cell by osmotic lysis.

Death by apoptosis is faster than osmotic lysis and is probably the main mechanism involved in killing of target cells by Tc cells.

* FAS: factor for apoptotic signal.

Innate



N.B.: Natural Killer cells (NK cells) are cells of the innate immune system which are capable of killing target cells using the same mechanisms as Tc cells. However, certain important differences exist between NK cells and Tc cells:

- 1- NK cells exhibit a natural ability to recognize and kill abnormal cells, such as virus-infected cells or tumour cells, without need for antigen-specific-MHC activation as required by T cells. Thus, they are important in the early stages of infection, before development of the acquired immune response.
- 2- NK cells do not show specificity to cells bearing certain antigen. In other words, the same NK cell can kill a cell infected with a certain virus, and go on to kill another cell infected by another virus, or even a tumour cell.
- 3- Sometimes antibodies can help NK cells to recognize abnormal cells. This mechanism is called antibody-dependent cellular cytotoxicity (ADCC) (described later with humoral immunity).

ADCC is

≠ opsonization

Cell mediated immunity (CMI) is a very important term related to T cell functions. It is applied to defensive reactions that are mediated primarily by activated T lymphocytes and macrophages. It is important in defence against intracellular pathogens, such as viruses, some bacteria and also against fungi. The two main effector functions of CMI are the cytotoxicity mediated by Tc cells and the macrophage activation and inflammation mediated by Th1 cells.

Specific } Non specific
کسین لجرے میں } کسین لجرے میں

Chapter 15

CYTOKINES = جزيئات خلية

Definition

Cytokines are peptide or glycoprotein mediators that are produced by cells of the immune system and have an effect on the behaviour and properties of many cells. Although T cells are the major source of cytokines, many other cells can produce them, and they have a wide range of functions, extending beyond the immune system (e.g. wound healing).

MCQ

Many different and overlapping names have been given to the various cytokines:

- Cytokines produced by lymphocytes are often called **lymphokines**.
- Many cytokines are given the name interleukin (IL), followed by a number (eg. IL-2).
between leukocyte & Ptn
- **Chemokines** are cytokines that are involved in the migration and activation of cells, especially phagocytic cells. → *neutrophils + monocyte*
- **Interferons** are cytokines capable of inducing body cells to resist viral replication, but they have other important functions, as well.
- *monokines from macrophage + monocyte*

General Characteristics of Cytokines

1. They are highly potent, often acting at very low concentrations.
2. They are not specific to antigens that induce their production.
3. They act through high-affinity cell surface receptors.
4. Their action is transient.
5. They act mainly in an autocrine manner (affecting the cell which produced them) or in a paracrine manner (affecting cells close by).
6. They are pleiotropic i.e. the same cytokine may have multiple effects.
7. Different cytokines may have the same activity redundancy.
8. They may act sequentially network interaction. They can also act together and increase the effect of one another synergy or act as antagonists.

More than 100 cytokines have been identified so far. Classifying cytokines is difficult because many are produced by more than one cell type and many have overlapping actions. (Fig. (20) shows a schematic overview of the most important cytokines. They can be classified into three main groups: **3 grs**

1. Cytokines that Mediate and Regulate Innate Immunity

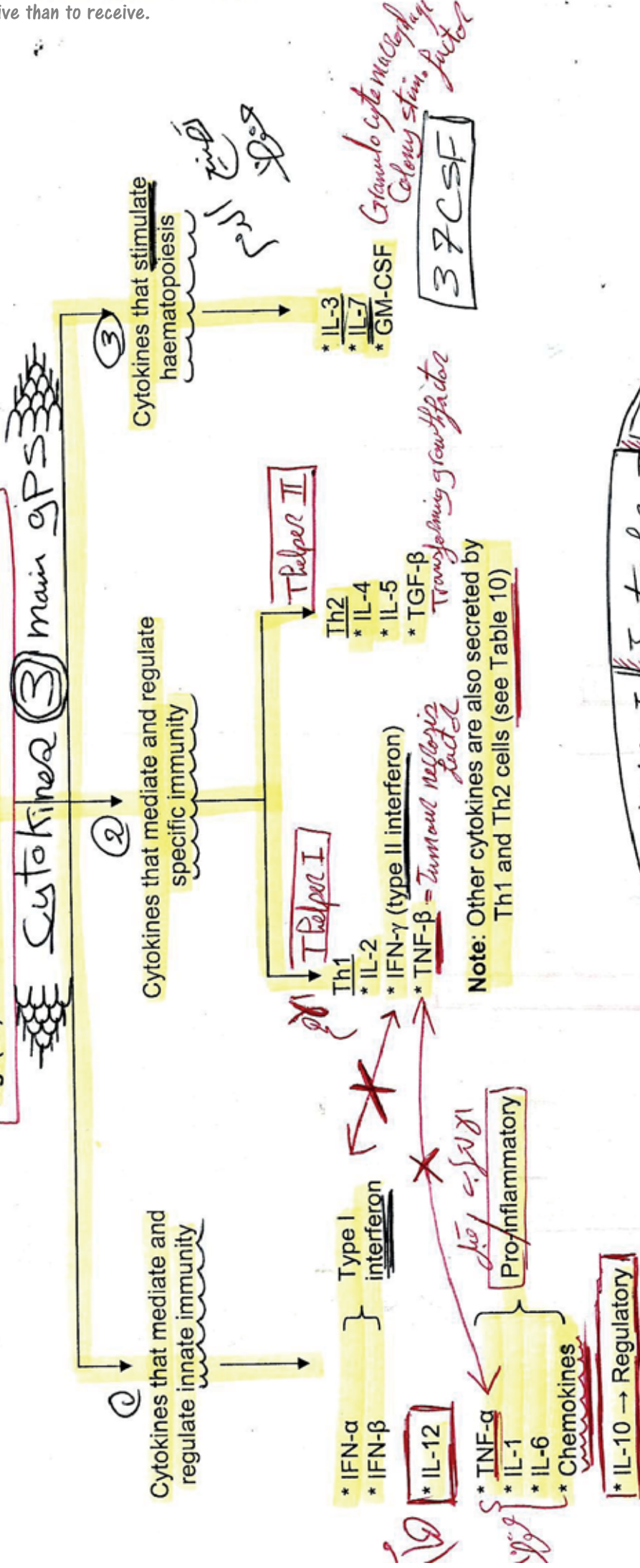
These cytokines are produced mainly by cells of the innate immune system and their actions serve to mediate innate immunity. However, some of them are produced by other cells as well and their actions can also affect cells of the specific immune system.

Type I Interferon (IFN)

- It is very important in viral infections. → *stimulate their production*
- It includes IFN-α and IFN-β.
- IFN-α is produced mainly by monocytes/macrophages.
not only

دکتر عزیز

Fig. (20): Schematic overview of important cytokines.



- IFN- β is produced mainly by fibroblasts.
- Many other cell types also produce both.
- The most important stimulus for production of type I IFN is viral infection.
- Actions:

1. Inhibition of viral replication: Type I IFN causes cells to synthesize a number of enzymes that interfere with the translation of viral mRNA. This antiviral action is mainly paracrine, meaning that a virally-infected cell secretes IFN to protect neighbouring cells not yet infected. A cell that has responded to IFN is resistant to any viral infection and is said to be in an anti-viral state.

2. Activation of NK cells: This is very important early in the course of infection, before the onset of the specific immune response.

3. Increased expression of MHC I molecules: This leads to more recognition of viral peptides and more efficient killing of virally-infected cells by CD8 Tc cells.

4. Inhibition of cell proliferation. $\rightarrow$ prevent cell growth e.g. in tumours

Note: The first three actions of type I interferon act together to eradicate viral infections, while the fourth action is important against tumours.

IFN- γ is called type II interferon and is mentioned later. $\rightarrow$ specific

Interleukin-12 (IL-12)

- It is produced mainly by monocytes/macrophages when stimulated by bacterial constituents.
- Actions:
 1. It increases the cytotoxic activity of NK cells as it stimulates NK cells to secrete IFN- γ .
 2. It promotes the differentiation of Th cells into Th1 cells. These, in turn, produce IFN- γ which activates macrophages.

Thus, IL-12 provides an important link between macrophages and NK cells and also between innate immunity and specific immunity (Fig. 21).

Tumour Necrosis Factor- α (TNF- α)

- It is the principal mediator of innate immune response against Gram-negative bacteria.
- It is produced mainly by monocytes/macrophages in response to bacterial lipopolysaccharide (endotoxin). It is also produced by Th1 cells.
- Actions:
 1. Small quantities of TNF- α act locally to recruit and activate neutrophils and other cells to combat bacterial infections, especially those caused by Gram-negative bacteria.
 2. In large quantities, TNF- α enters the blood stream, causing systemic effects such as fever, shock and even death. Many of the manifestations of septic shock are actually caused by oversecretion of TNF- α .
 3. TNF- α induces the production of IL-1, and both cytokines have similar pro-inflammatory actions:
 - Production of fever.
 - Promotion of local inflammation.
 - Induction of synthesis of acute phase proteins.

* Why not used frequently in Tumours $\Rightarrow$ Large quantities

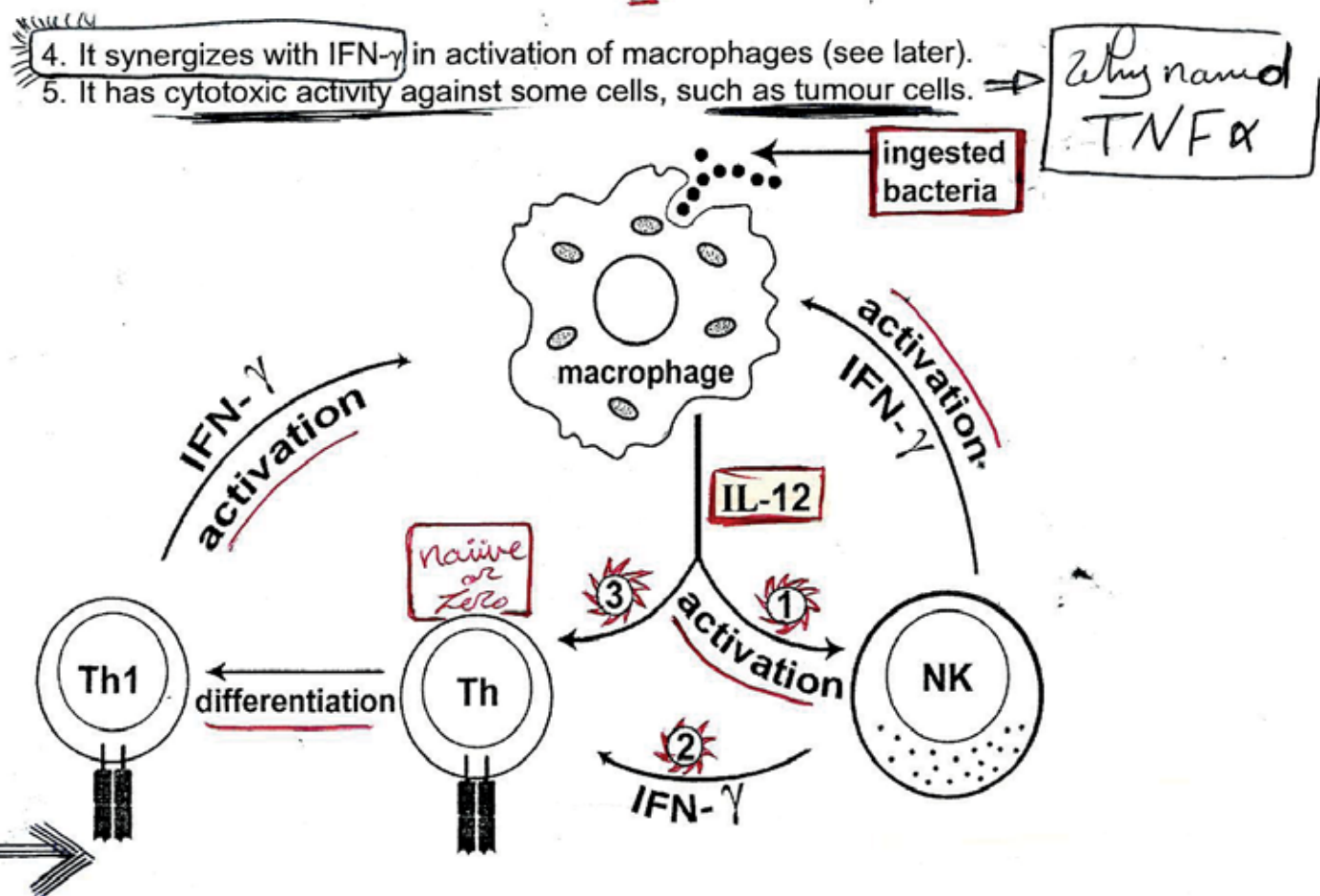


Fig. (21): Actions of IL-12.

Interleukin-1 (IL-1)

- It is considered an important mediator in the host inflammatory response in innate immunity.
- It is produced mainly by monocytes/macrophages, triggered by bacterial lipopolysaccharide.
- Actions: It has the same pro-inflammatory actions as $\text{TNF-}\alpha$.

Interleukin-6 (IL-6)

- It is produced by monocytes/macrophages as well as Th2 cells and others.
- Actions:
 1. It has pro-inflammatory actions.
 2. It is a growth factor for B cells.

The chemokines

These are chemotactic cytokines that are capable of stimulating leucocyte movement in a certain direction. Examples:

- Interleukin-8: produced by monocytes/macrophages and is chemotactic for neutrophils.
- Eotaxin: chemotactic for eosinophils. *eo = eosinophil + tax + in (ptn)*

($\text{TNF-}\alpha$, IL-1 , IL-6 and the chemokines are known as the **pro-inflammatory cytokines**).

fever inflammatory reaction

Interleukin-10 (IL-10)

- It is produced by macrophages, Th2 cells and others.
- It is considered a regulatory cytokine, inhibiting certain cells.

II. Cytokines that Mediate and Regulate Specific Immunity

These cytokines are produced mainly by Th cells and mediate their actions. Some are produced by Th1 cells, some by Th2 cells and some by both (Table 8).

عبدالمنعم محمد الشبان

Table (8): Comparison between Th1 and Th2 cells:

	Th1	Th2
Cytokines produced	IL-2 IFN-γ TNF-α and β	IL-4 IL-5 IL-6 IL-10 TGF-β
	GM-CSF IL-3	GM-CSF IL-3
Development promoted by	IL-12 IFN-γ	IL-4
	Large doses of antigen	Small doses of antigen
Development inhibited by	IL-4 IL-10	IFN-γ
Promote	Cell-mediated immunity	Humoral immunity

Interleukin-2 (IL-2)

- It is produced by activated Th1 cells.
- It is mainly known as an autocrine and paracrine growth factor for T cells.
- Actions:
 - It promotes proliferation of T cells, and B cells.
 - It promotes cytokine production by T cells.
 - It activates NK cells so that their killing ability is enhanced. NK cells activated by IL-2 become lymphokine-activated killer cells (LAK cells).

IL2 + NK $\rightarrow$ (LAK) define
Lymphokine activated killer cells

Interferon- γ (IFN- γ) type II Interferon = Immune interferon

- It is called type II interferon or immune interferon.
- It is produced mainly by Th1 cells and is considered the hallmark of Th1 cells, i.e. production of IFN- γ defines a Th cell as a Th1 cell.
- It is also produced by NK cells.
- Actions:
 - Activation of macrophages (It synergizes with TNF- α in this action):
 - It promotes fusion of phagosomes containing the bacteria to lysosomes containing anti-bacterial substances.



- It induces synthesis of nitric oxide and other bactericidal substances. + *oxygen radical*
- It induces macrophages to secrete their cytokines.
- 2. It increases the expression of MHC I molecules, leading to better killing of target cells by Tc cells.
- 3. It increases the expression of MHC II molecules on APCs, leading to better presentation of antigens to Th cells.
- 4. It promotes the development of Th1 cells and inhibits the development of Th2 cells.
- 5. It activates NK cells.

*develop Th₁
Inhibit Th₂*

Tumour Necrosis Factor- β (TNF- β)

- It is produced by Th1 cells.
- It has actions similar to TNF- α (see before).

Th₂

Interleukin-4 (IL-4)

- It is produced by Th2 cells and is the hallmark of these cells, i.e. production of IL-4 defines a Th cell as a Th2 cell.
- It is also produced by NK cells and mast cells.
- Actions:

1. It helps activation and growth of B cells.
2. It promotes production of IgE.
3. It promotes growth and function of mast cells and eosinophils.

(The above three actions promote the development of type I hyper-Sensitivity)

4. It promotes the development of Th2 cells and inhibits the development of Th1 cells.
5. It suppresses the synthesis of the pro-inflammatory cytokines.

*develop Th₂
Inhibit Th₁*

allergic sensitivity → How IL4 Cause Type I hypersensitivity reaction

*IFN γ → Th₁
IL-4 → Hallmark → Th₂*

Interleukin-5 (IL-5) ⇒ *eosinophils growth + differentiation*

- It is produced mainly by Th2 cells.
- Actions:
Its main function is promoting growth and differentiation of eosinophils, thus, playing an important role in allergic diseases and control of helminthic infections.

Transforming growth factor- β (TGF- β) ⇒ *الفاكتور*

- It is produced by Th2 cells as well as macrophages.
- Actions:
 1. It was originally discovered as a growth factor that promotes wound healing.
 2. It is an immunosuppressive cytokine.

*① Immunosuppressor
② Promote wound healing
③ as growth factor*

III. Cytokines that Stimulate Haematopoiesis

These are cytokines that support the production of all blood cells, or particular blood cells:

Interleukin-3 (IL-3)

- It is produced by both Th1 and Th2 cells.
- It promotes formation of blood cells.



Granulocyte-monocyte colony stimulating factor (GM-CSF)

- It is also produced by Th1 and Th2 cells.
- It promotes development of granulocytes and monocytes.

* Interleukin-7 (IL-7) Bone marrow → T & B

- It is produced by thymic and bone marrow stromal cells.
- It promotes production of T and B cells.

* Therapeutic Uses of Cytokines

There is considerable interest in the possible use of cytokines as therapeutic agents, either to augment an immune response, or to inhibit inflammation (by using anti-inflammatory cytokines such as IL-10 and TGF- β). However, the administration of cytokines in therapeutic doses may lead to toxicity.

Examples of Therapeutic Uses

Interferon- α (IFN- α)

- Has shown success in treatment of viral hepatitis.
- Is being tested as a possible treatment for many malignancies especially lymphomas and leukaemias.

Interleukin-2 (IL-2)

Experimentally, its administration to normal or immunodeficient mice enhances immune responses, but use in humans is limited by severe toxic side effects.

Granulocyte-monocyte colony stimulating factor (GM-CSF)

They can be used to treat cases of leucopenia and bone marrow depression.

* Give an account on Therapeutic uses of Cytokines
+
اول الشاير

Chapter 16

THE HUMORAL IMMUNE RESPONSE

Antibody $\neq$ Humoral

The main function of the humoral immune response is the destruction of extracellular pathogens (e.g. extracellular bacteria) and the prevention of spread of intracellular pathogens (e.g. intracellular bacteria and viruses) as they move from cell to cell through the extracellular fluids. This is achieved by molecules known as antibodies or immunoglobulins (Igs).

The first stage in the production of antibodies is activation of the resting naïve B-lymphocytes and their differentiation into antibody-secreting plasma cells. Certain receptors (Fig. 22) are expressed on the surface of the B cells and are essential for their activation. These are:

* Stages of production
① activation naïve B
② differentiation
③ expressing receptors

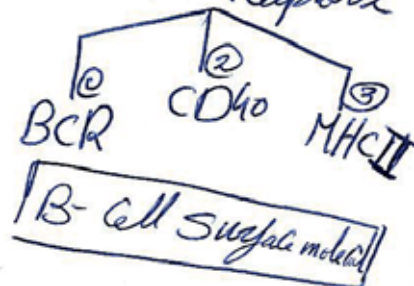
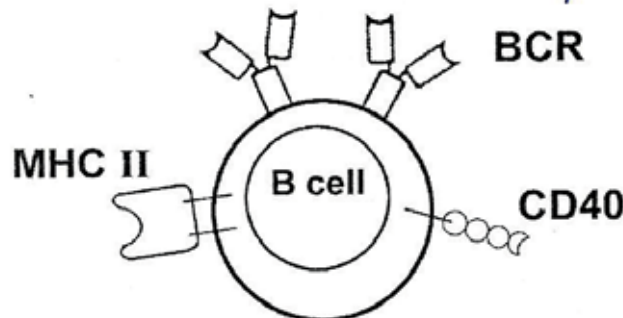


Fig. (22): B cell surface molecules.

not TCR $\Rightarrow$ there was $\alpha + \beta$ polypeptide

a- **B cell receptor (BCR)**: This is a membrane-bound antibody molecule, also called surface immunoglobulin, that acts as an antigen receptor. All BCRs on a single B cell are of identical specificity and a B cell that binds antigen through this molecule will then secrete antibodies of the same specificity. Immature B cells express only IgM on their surface, whereas mature B cells bear both IgM and IgD.

b- **CD40**: These molecules are essential for the interaction between B and T cells.

c- **MHC II**: These molecules are essential for antigen presentation to T helper cells. + MHC I

Sequence of events in activation of naïve B cells: (Fig. 23)

B cell activation is a multi-step process that occurs as follows:

- The mature naïve B cell leaves the blood stream and enters a secondary lymphoid organ (e.g. lymph node). Upon entry, the B cell migrates into a region rich in T cells.
- In the absence of its target antigen, the B cell traverses this region rapidly and eventually re-enters the circulation.



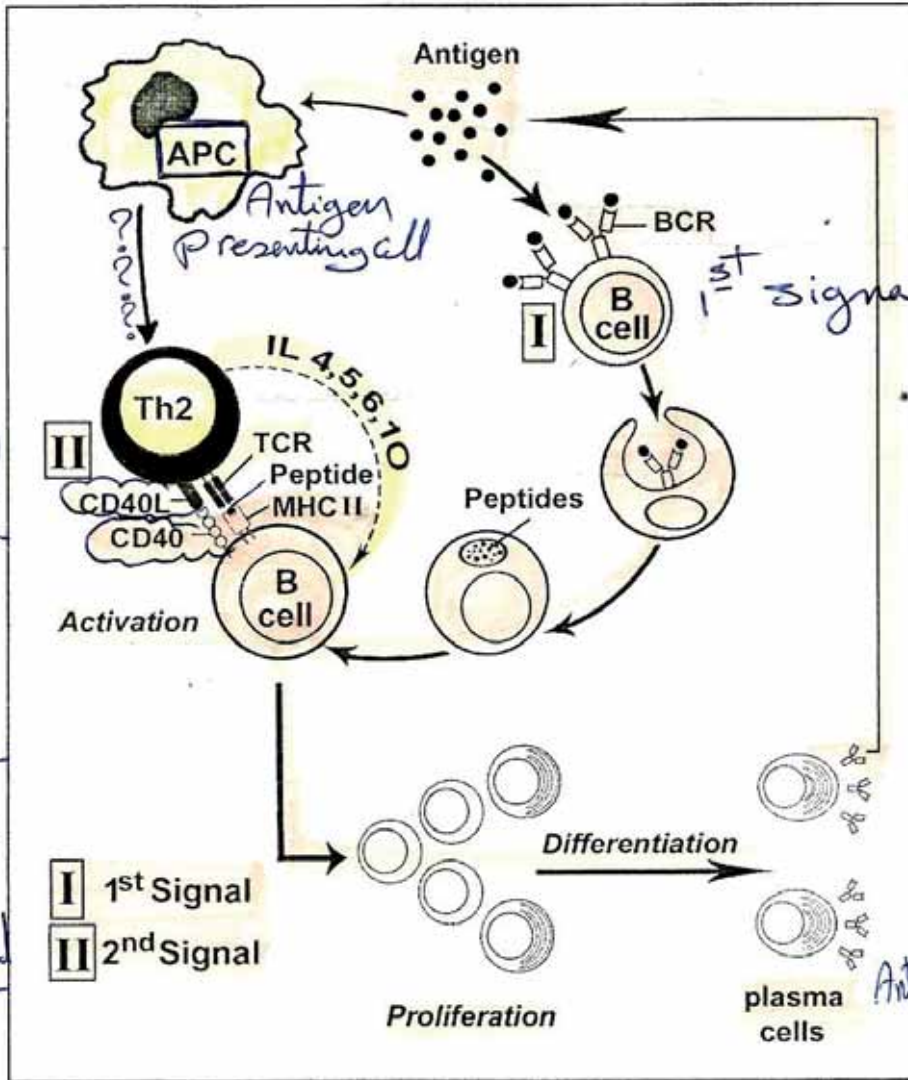


Fig. (23): Activation of naïve B cells.

2nd signal
 2nd signal
 Th must be activated to give 2nd signal

- In the presence of its target antigen, the B cell binds its specific antigen via the surface Igs (BCR). Those B cells that have bound antigen are selectively trapped before leaving the T cell zone, where full activation of the B cells occurs.
- To become fully activated, naïve B cells must receive 2 signals:
- The first signal is delivered by binding of the antigen to BCR.
 - The second signal (called accessory or co-stimulatory signal) is delivered by activated helper T cells (mainly Th2). To receive this signal, the B cell engulfs the bound antigen, degrades it into peptides and presents these peptides on the cell surface in association with MHC class II molecules. The peptide-MHC class II complex can then be recognized by antigen-specific helper T cell. This triggers the T cell to produce:
- CD40 ligand (CD40L), which is a T cell surface molecule that binds to the B cell surface molecule CD40.
 - IL-4, IL-5, IL-6 and IL-10, which are B cell stimulatory cytokines.

MHC

Th2
 B-cell different.
 factors

Antibody Secreting

- The B cell and T cell are now bound together; the B cell has received both signals (antigen-derived and T cell-derived). These stimulate the B cell to become a lymphoblast (**activation**) and to divide repeatedly (**proliferation** or **clonal expansion**). Its progeny (daughter cells) become effector cells, which are antibody-secreting plasma cells (**differentiation**). IgM is first secreted, then the B cell can switch to the production of another isotype of the same immunologic specificity.
- Some B-lymphocytes do not undergo this terminal differentiation. Instead, they become **memory cells** which are long-lived and the major cells responsible for immunological memory. This sequence of events is known as the **primary immune response**. Most antigens require this form of B cell - T cell collaboration to initiate an immune response and are, therefore, termed **thymus-dependent (TD) antigens**.
- Some antigens, such as bacterial polysaccharides, can activate B cells directly in the absence of T cell help. These antigens are called **thymus-independent (TI) antigens**. In such cases, B cell activation is delivered by the antigen only. TI responses provide an early and specific antibody response against many important bacterial pathogens without the need for T cell activation. However, only IgM is produced and the B cell cannot switch to production of other isotypes. In addition, memory cells are not produced.

IMMUNOGLOBULINS

After the proliferation of the activated B cells and their differentiation into antibody-secreting plasma cells, surface immunoglobulins (BCR) disappear, and the secretion of large amounts of antibody begins.

Define?

Antibodies or immunoglobulins (Igs) are glycoproteins that bind specifically to the antigen that induced their formation. In the blood, most of the immunoglobulins are present within the gamma globulin fraction of plasma proteins. They are also present in the extravascular compartment, e.g. lymph and tissue fluids.

GAMED

There are 5 classes or isotypes of Igs, namely, IgG, IgA, IgM, IgE and IgD. Within certain classes, there are subclasses that show slight differences in structure and function, e.g. IgG1, IgG2, IgG3 and IgG4.

Antibody Structure: (Fig. 24)

The basic structure is common to all classes of Igs. An antibody molecule is roughly Y-shaped and consists of 2 identical light (L) and 2 identical heavy (H) polypeptide chains linked together by disulphide (S-S) bonds.

The light chains → 200 a.a. + 25 kDa

Each light chain is composed of approximately 200 amino acids and has a molecular weight of about 25 kDa. They are one of two types, kappa (κ) or lambda (λ). Both types can be found in all classes of Igs, but only one type is found in one antibody molecule.

TD = IgM → class switch
 TI = IgM only no class switch
 @ majority of antigens e.g. Ptn
 @ few antigens
 @ don't require Th cell help
 @ no memory cells
 @ due to memory → due to cytokines
 @ NOT cytokines

circ Light chain

The heavy chains

5 γ GAMED $\rightarrow$ 400 a.a. @ 50-75 kDa

Each heavy chain is composed of approximately 400 amino acids, which is twice the number in the light chain, and has a molecular weight of 50-75 kDa, which is also approximately twice that of the light chain. The heavy chains determine the isotype of an Ig. There are 5 main types: gamma (γ), alpha (α), mu (μ), delta (δ) and epsilon (ϵ), corresponding to the 5 isotypes of Igs IgG, IgA, IgM, IgD and IgE respectively. The two heavy chains are joined by a number of S-S bonds in the region known as the hinge region.

class = isotype

Hinge region = region of 2 heavy chain joined by S-S bonds

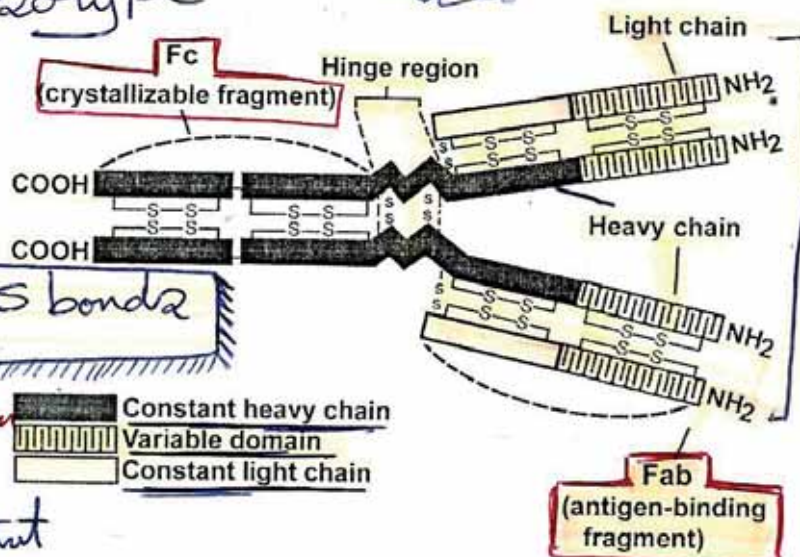


Fig. (24): Basic structure of immunoglobulin molecule.

Domains = regions of Light & Heavy chains $\rightarrow$ 110 a.a.

- The light and heavy chains are subdivided into regions or domains, each formed of around 110 amino acids.
- These regions are:
 - Variable regions or domains, which show a wide variation in amino acid composition.
 - Constant regions or domains, which demonstrate a much more uniform (constant) amino acid sequence.
- The light chain consists of one variable (VL) and one constant domain (CL). The heavy chain consists of one variable (VH) and 3 or 4 constant domains (CH₁, CH₂, CH₃, CH₄).

specific to antigen $\rightarrow$ The amino (NH₂)-terminal of the antibody molecule: It is formed of the variable domains of heavy and light chains (VH, VL). They constitute the antigen-binding sites. Since the antibody has two identical light chains and two identical heavy chains, each antibody will have two identical antigen-binding sites. This allows the antibody molecule to cross-link antigens.

specific to class $\rightarrow$ The carboxyl (COOH)-terminal of the antibody molecule: It is formed of the constant domains of both heavy chains. It is the same for all members of the same isotype, and determines the functional properties of a particular isotype.



★ Hypervariable regions

The variability in amino acid sequence in the variable domains of light and heavy chains is not spread evenly over their entire length but is restricted to short segments. These segments show considerable variation and are termed hypervariable regions. The hypervariable regions of heavy and light chains are folded and brought together, creating a single hypervariable surface or paratope. The paratope is the antigen-binding site; it is complementary to and interacts with the epitope of the antigen (Fig. 25).

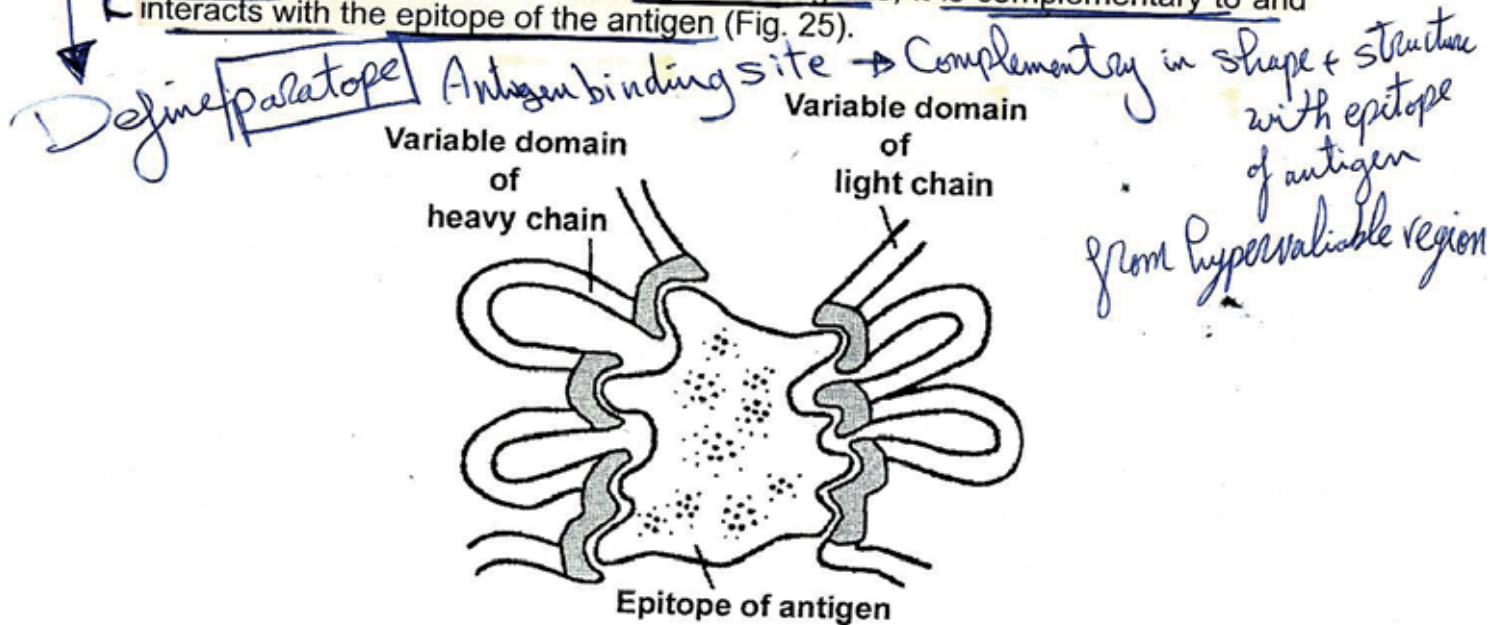
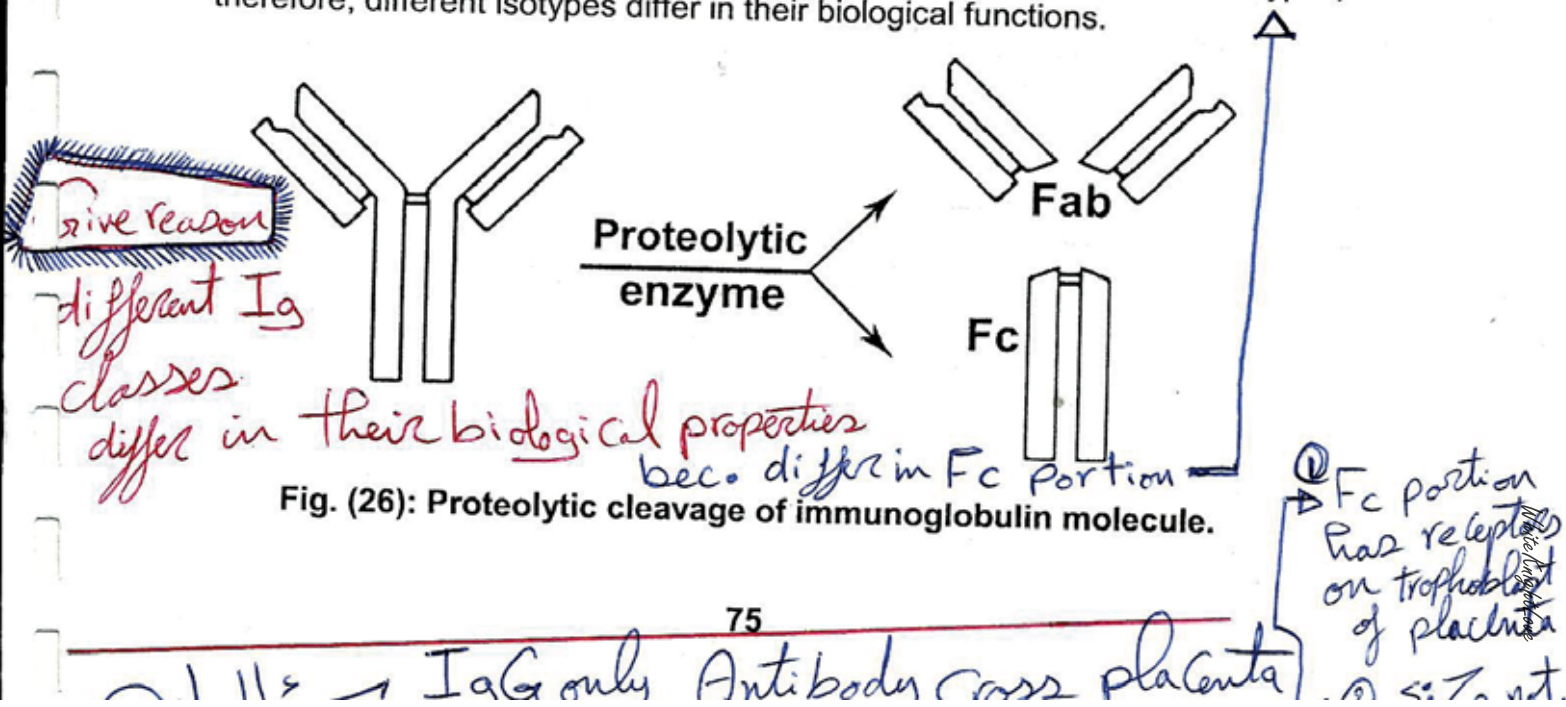


Fig. (25): Hypervariable region.

Proteolytic cleavage: (Fig. 26)

If an antibody molecule is treated with a proteolytic enzyme, peptide bonds in the hinge region are broken. This produces:

- Two identical **Fab** fragments (fragment antigen binding) which carry the antigen-binding sites, and
- One **Fc** fragment (fragment crystallizable, because it crystallizes easily) which is involved in the biological activities of the antibody molecule such as complement fixation, placental transfer and attachment to various cells with Fc receptors. The Fc fragment differs in antibodies of different isotypes; therefore, different isotypes differ in their biological functions.



Functions of Antibodies

not killer only Binding

There are several ways in which antibodies contribute to immunity:

- 1. Agglutination:** Binding of antibodies to a particulate antigen (e.g. bacteria) results in clumping of the pathogen which prevents its dissemination and stimulates its removal by other mechanisms (e.g. phagocytosis).
- 2. Neutralization:** Antibodies can inhibit the infectivity of a pathogen (viruses or bacteria) or the toxicity of a toxin molecule by binding to them, thereby preventing their attachment to their specific receptors on their target cells.
- 3. Opsonization:** Phagocytic cells have Fc receptors on their surface, that can recognize and bind Fc portion of antibody molecules coating a pathogen. This facilitates the engulfment and subsequent intracellular killing of the pathogen by the phagocytic cells (Fig. 27).

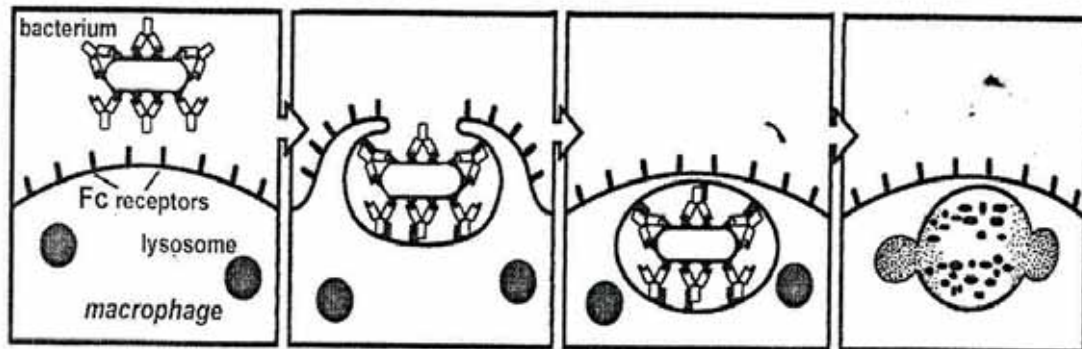


Fig. (27): Antibody-mediated opsonization.

- 4. Complement activation:** Antibodies bound to the surface of a pathogen may activate proteins of the complement system. Some of these complement proteins become deposited on the pathogen and also bind to complement receptors on phagocytes, favouring the uptake and destruction of the pathogen by the phagocyte. This is another example for opsonization. Other complement proteins may lyse the pathogen directly by forming pores in its membrane (Fig. 28).

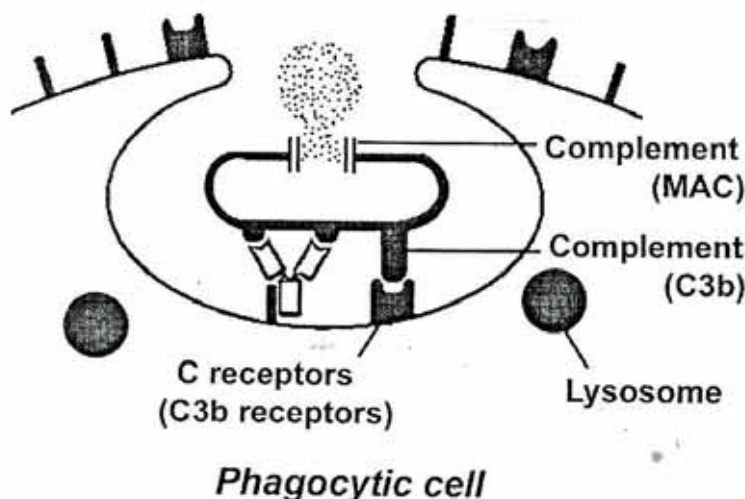
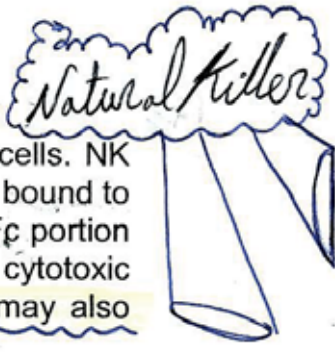


Fig. (28): Complement-mediated opsonization and bacterial cell lysis.



5. Antibody-dependent cell-mediated cytotoxicity (ADCC): (Fig. 29)

It is the destruction of antibody-coated cells by natural killer (NK) cells. NK cells possess receptors for the Fc portion of antibodies. An antibody bound to an antigen on a target cell can also bind to the NK cell through its Fc portion facilitating adhesion of the NK cell to the target cell and triggering its cytotoxic activity. Other cells possessing Fc receptors, e.g. macrophages, may also exert ADCC.

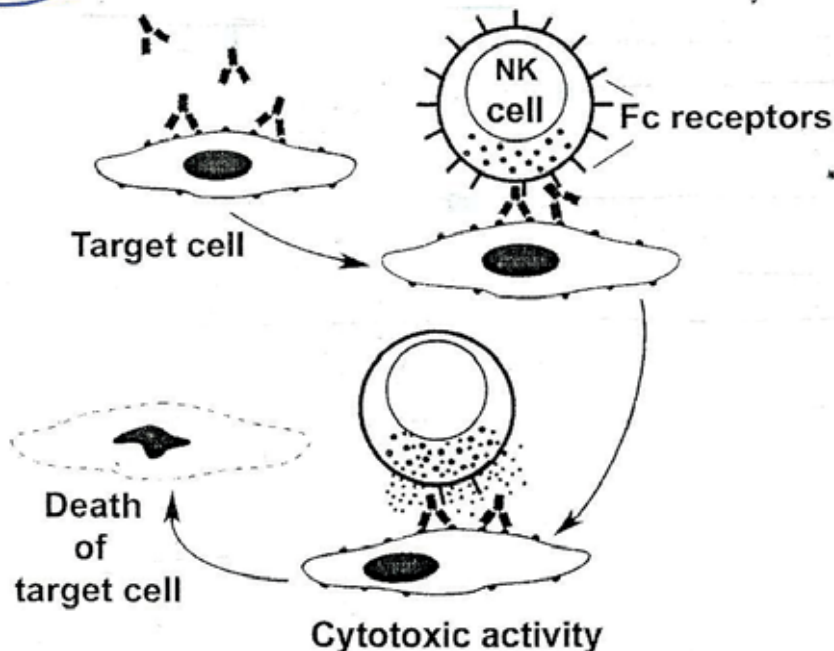


Fig. (29): Antibody-dependent cell-mediated cytotoxicity (ADCC).

N.B.: The type of the antibody (isotype) produced determines which effector mechanism occurs in a particular immune response.

Immunoglobulin Classes (Isotypes)

I. Immunoglobulin G (IgG)

- IgG is composed of a single basic unit (monomer).
- There are 4 subclasses (IgG₁-IgG₄) based on H chain differences.
- It is the principal isotype in blood (75% of circulating Igs) and extracellular fluids.
- It is the major antibody of the secondary immune response.
- Placental transfer: IgG interacts with Fc receptors in the placenta and is, therefore, the only Ig that can pass the placental barrier to the foetal circulation. This provides passive protection to the newborn during the first few months of life.
- Anti-Rh antibodies are of the IgG class.
- Biological activities:
 - Neutralization.
 - Opsonization.
 - Complement activation.
 - ADCC.

Handwritten notes:
 ⊕ monomeric basic unit
 ⊕ Majority 75%
 ⊕ 2nd immune response
 ⊕ pass placenta

Handwritten notes:
 if RH-ve ♀ ⊕ RH+ve ♂
 → erythroblastosis fetalis
 in 2nd baby

⊕ don't pass placenta ⊕ major Antibody freely you have received, freely give. ⊕ only Antibody to T1 antigen



II. Immunoglobulin M (IgM)

- IgM is composed of 5 basic units (pentamer) held together by disulphide bonds and a single J (joining) chain (Fig. 30).
- Because of its large size, IgM is mainly confined to the blood (8-10% of circulating Igs).
- It is the major antibody of the primary immune response.
- It is the only antibody made to thymus-independent antigens (e.g. ABO blood group antigens of human RBCs). **TI = cho in nature**
- IgM cannot cross the placenta; therefore, its presence in the newborn blood indicates intrauterine infection. **no ABO incompatibility**

Biological activities:

- Agglutination.
- Complement activation.

IgM is the most efficient agglutinating and complement-fixing antibody.

IgM is found on the surface of B cells forming BCR.

IgG only
IgM only

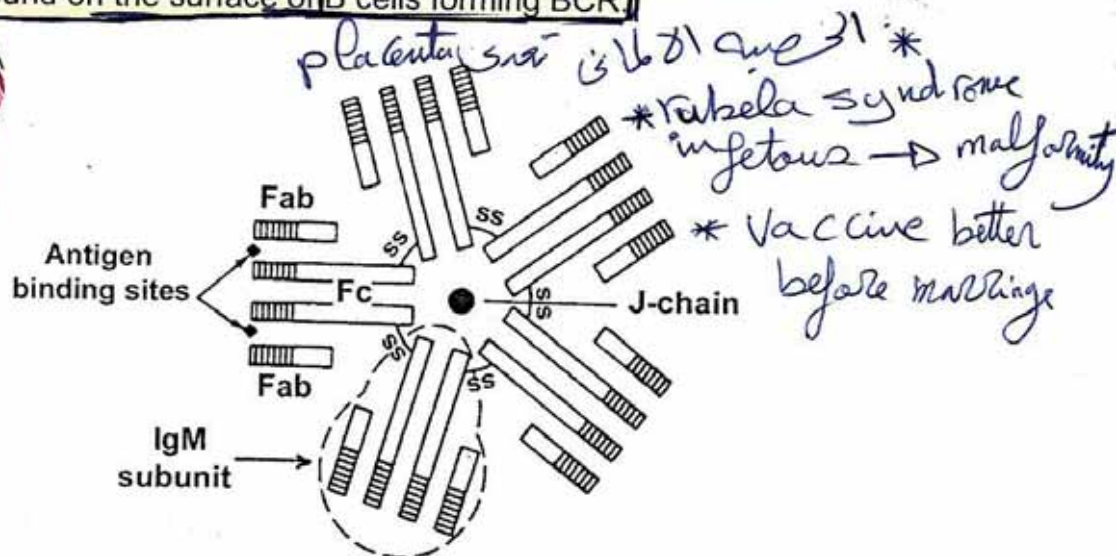
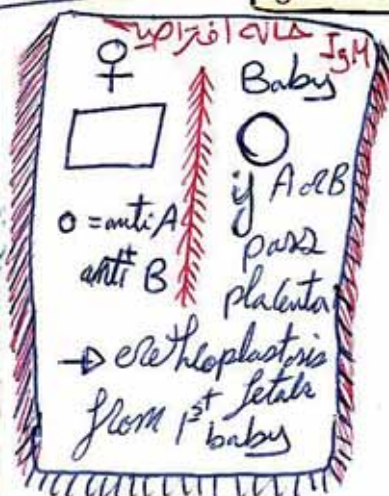


Fig. (30): Immunoglobulin M (IgM) molecule.



III. Immunoglobulin A (IgA)

This class of immunoglobulins exists in two forms:

1. Monomeric form (serum IgA), which is found in serum. It represents about 15-20% of total serum immunoglobulins and its function is uncertain.
2. Dimeric form (secretory IgA), which is produced by the submucosal plasma cells and is found in the mucosal secretions (saliva, tears, colostrum, respiratory, GIT and genitourinary secretions). The dimeric form of secretory IgA is composed of two basic units, a short peptide chain (J chain) that joins the two units together, and a secretory component. The secretory component is synthesized by local mucosal cells. It facilitates the passage of IgA through the epithelial cells and protects the molecules from proteolytic digestion (Fig. 31).

Secretory IgA provides local immunity at the mucosal surfaces. Its main function is neutralization as it prevents attachment of organisms to mucosal surfaces.

J chain makes IgM @ IgA don't make opsonization

Macrophage Fc → need a cell → monomeric so only

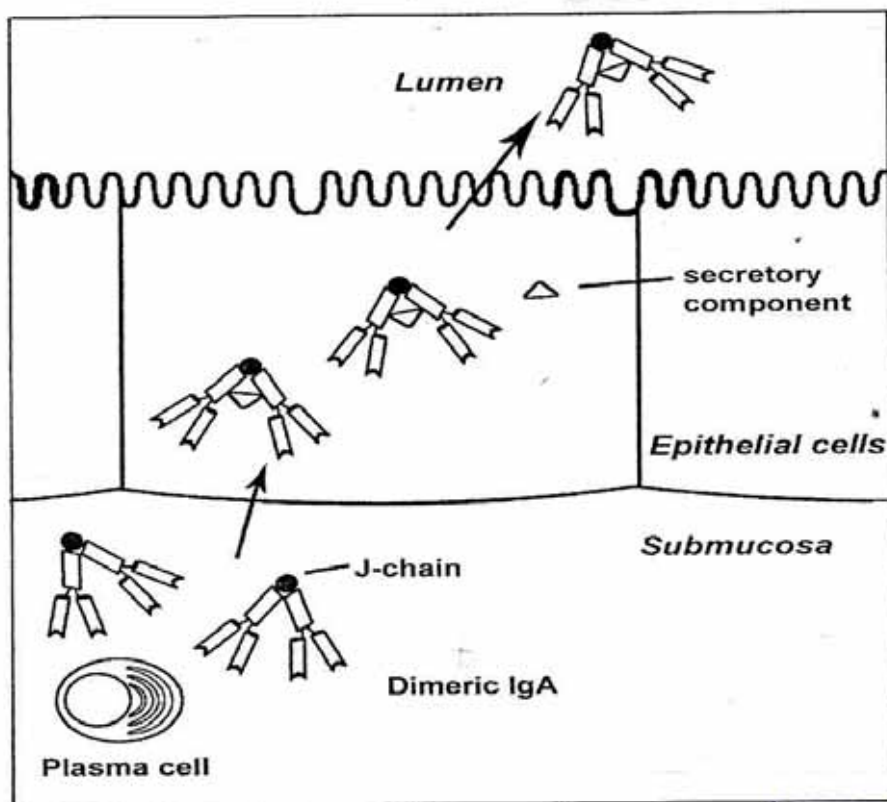


Fig. (31): Secretory IgA.

$IgM + IgD$

IV. Immunoglobulin D (IgD) $\oplus 1\% \oplus monomer$

- IgD exists as a monomer.
- It accounts for less than 1% of circulating antibodies.
- It is found on the surface of mature B cells where it acts as an antigen receptor (BCR).

on mature B cells BCR

V. Immunoglobulin E (IgE)

- IgE exists as a monomer.
- It is present in trace amounts in serum.
- It is also present attached to Fc receptors on mast cells and basophils and plays an important role in type I hypersensitivity reactions.
- IgE binding to Fc receptors on eosinophils may be important in immunity to parasitic worms, as it triggers eosinophils to release toxic substances on the surface of the parasite.

no opsonisation on eosinophils $\rightarrow$ toxic
Type I Hypersensitivity on mast + basophils

Immunoglobulin Class Switching (Isotype Switching): (Fig. 32)

During the immune response, plasma cells switch from producing IgM to produce IgG or other immunoglobulin classes. Class switching is mediated by a change in the constant domains of the heavy chain (CH). There is no alteration in the L chain or the variable domain of the heavy chain (VH), so that the immunoglobulin produced later (IgG, IgA or IgE) has the same specificity as the original IgM but differs in the biological characteristics.

TD
Not
TI

Plasma cell $IgM \rightarrow IgG$
Switch $\uparrow$
production by change in Constant Domain heavy chain

* Class switching is dependent on cytokines released from T cells. Under the effect of IL-4 alone the expanded B-cell clones can differentiate and mature into IgE-secreting cells. TGF- β (transforming growth factor- β) encourages cells to switch their Ig class to IgA, and then IL-5 augments IgA production by these cells. High rates of IgG secretion occurs from plasma cells under the combined influence of IL-4, IL-5 and IL-6.

class switching depend on

Cytokines of T cells

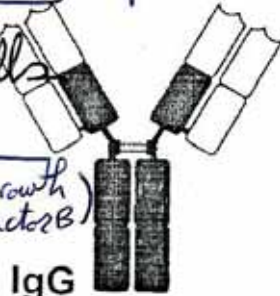
IL-4 alone $\rightarrow$ IgE

TGF- β (transforming growth factor- β)

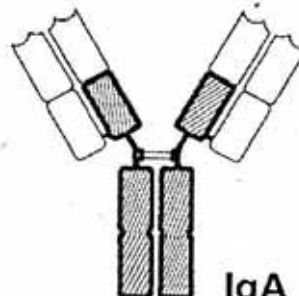
Ig $\rightarrow$ IgA

then IL-5 Augment IgA production

$\uparrow$ High rates IgG = Influence IL-4 $\oplus$ IL-5 $\oplus$ IL-6 Combined



IgG



IgA

Fig. (32): Immunoglobulin class switching.

Primary and Secondary Antibody Response: (Fig. 33)

Primary antibody response occurs when an antigen is introduced for the first time. (There is a lag (induction) period during which antibody not detected (7-10 days). This is the time needed for B cells to undergo activation, proliferation and differentiation into antibody-secreting plasma cells. The isotype produced is mainly IgM. With time, various mechanisms turn off the response e.g. removal of the antigen. Then antibody production is stopped and there is rapid decline in its concentration to undetectable levels.

Secondary antibody response occurs on subsequent exposure to the same antigen. Memory cells (generated in the first exposure) are readily activated and antibodies can be detected after a short lag period. IgG is the main isotype produced. The antibody level is 10 times greater than during the primary response and is maintained at high levels, falling slowly over a period of months. The response can be boosted to higher levels by further exposures. For this reason, most vaccines are given in more than one dose (booster doses).

Give Reason

most vaccine given in multiple doses to boost response of immunity to higher level

Table (9): Comparison between primary and secondary antibody response:

	Primary response	Secondary response
Induction (lag) period	Long (7-10 days)	Short (few hours to few days)
Antibody level	Low	High (10 times greater)
Duration	Short (antibodies decline rapidly)	Long (months)
Ig class	Predominantly IgM	Predominantly IgG
Memory cells	Absent	Present
Definition	first exposure	subsequent exposure

$\rightarrow$ Curve + lag + rise

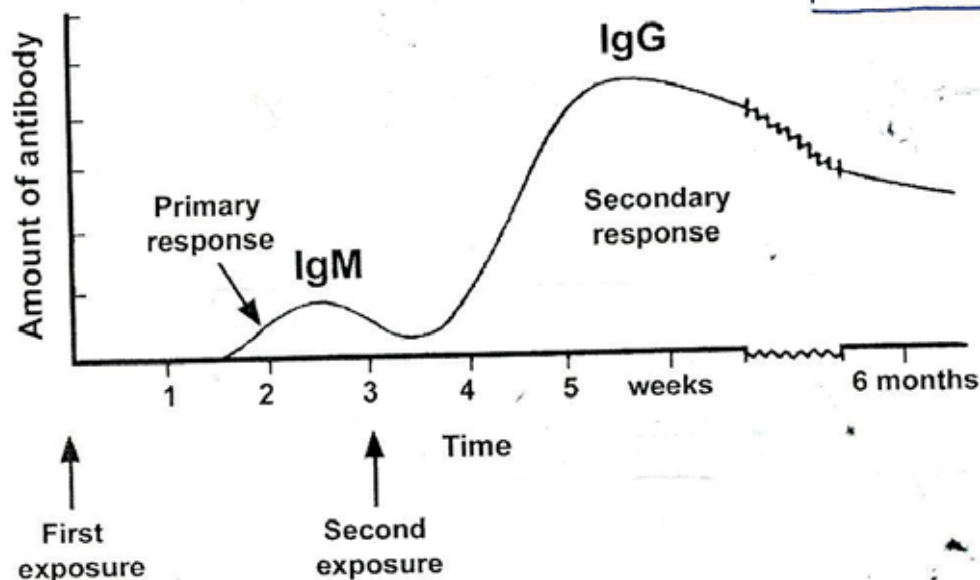


Fig. (33): Primary and secondary antibody response.

Ch 13 Fig 13

Heterophil Antibodies

Because of the similarity that may be found between different antigens, antibodies produced in response to an antigen may cross-react with another one. Such cross-reacting antibodies are called "heterophil antibodies".

if Antigen same epitopes → Antibodies of epitopes cross react each other

Monoclonal Antibodies

These are highly specific antibodies against a single epitope produced by a single clone of B cells. They can be artificially produced to be used in diagnosis and therapy.

1953 Nobel Prize not against antigen as whole

a) Diagnostic applications

Monoclonal antibodies are widely used in different kinds of serological reactions for antigen detection, e.g.:

1. Determination of lymphocyte markers (e.g. CD markers).
2. Detection of HLA antigens (tissue typing).
3. Detection and typing of viruses.
4. Hormonal assays.
5. Detection of tumour antigens.

b) Therapeutic applications

1. Antitumour therapy; the use of tumour specific monoclonal antibodies linked to cytotoxic drugs (magic bullet therapy).
2. Immunosuppressive therapy in graft rejection; the use of monoclonal antibodies against CD3 on T cells.
3. Treatment of drug toxicity e.g. digitalis. → *Cardiac arrhythmia*
4. Passive immunotherapy in some viral diseases.
5. Prevention of Rh incompatibility; the use of monoclonal anti-Rh D.

to neutralize digitalis toxicity

Define magic bullet therapy → monoclonal antibodies + toxic drugs in Antitumour

*Antibody not killer alone *

*But + Complement → bactericidal

Chapter 17

freely you have received; freely give.

humoral innate

In all body fluid except urine + CSF



- The complement system is a group of heat-labile proteins normally found in blood and tissue fluids (except urine and CSF).
- These proteins are termed complement factors because they are required to 'complement' the bactericidal effects of antibodies.
- They are mainly produced by the liver.
- The basic complement proteins are termed C_1 to C_9 , in addition to factor B, D and properdin and some complement regulatory proteins. Most of them are normally found in an inactive form. Activation of complement occurs through interaction of complement factors in a sequential manner one step after the other. The product of one reaction forms the enzyme for the next, and so on. This mode of activation is called complement activation cascade.
- When they become activated, some complement factors are split into a small fragment (a) which is considered a by-product, and a large fragment (b) which continues the activation process.

Complement Activation

There are 3 pathways for complement activation: the classical pathway, which is triggered by antibodies, and the lectin and alternative pathways which are initiated in the absence of antibody. The early steps in all pathways involve a series of cleavage reactions which end with the production of an enzyme, C_3 convertase, that splits the third complement component (C_3) into C_{3a} and C_{3b} . C_{3b} becomes attached to microbial surface and initiates the late steps of the complement cascade. Thus, the 3 pathways of complement activation differ in how they are initiated (early steps), but they share the late steps and also perform the same effector functions.

1. The classical pathway

This pathway is activated by antigen-antibody complexes (immune complexes). Only IgG or IgM can activate (fix) complement. The reaction starts by binding of the first complement component (C_1) to the Fc portion of the antibody molecule attached to the antigen (e.g. a bacterial cell). This causes activation of C_1 which acts as an enzyme that cleaves 2 other complement proteins, C_4 and C_2 , into small (a) and large (b) fragments. The resultant C_{4b} C_{2b} is the C_3 convertase of the classical pathway, that splits C_3 into C_{3a} and C_{3b} . C_{3b} adheres to the microbial surface to start the late steps of complement activation.

The late steps start by cleavage of C_5 into C_{5a} and C_{5b} . C_{5b} binds to the terminal complement components C_6 , C_7 , C_8 and C_9 sequentially to form a complex, called membrane attack complex (MAC). This complex ($C_{5b,6,7,8,9}$) forms a hollow cylinder that becomes inserted into the target cell membrane, allowing free passage of water and solutes across the membrane which leads to cell death (osmotic lysis).

N.B.: Activation of complement via the classical pathway occurs in the following order $C_{1,4,2,3,5,6,7,8,9}$.

$C_1 = 3$ fragment together = active

← small fragment = inactive

بما كس القوسه اي صفر كس
يفر كس
البيو

In absence of antibody

Mention C₃ Convertase in each Pathways

2. The lectin pathway:

This pathway is activated when a plasma protein, mannose-binding lectin (MBL), binds to mannose residues on microbial surface. MBL is structurally similar to C₁ and activates C₄. The subsequent steps are essentially the same as in the classical pathway.

3. The alternative pathway:

C₃ is abundant in plasma, and C_{3b} is produced continuously by its spontaneous cleavage. Although much of this C_{3b} is inactivated by hydrolysis, some attaches to the host cell surface or microbial surface.

The alternative pathway is triggered when C_{3b} becomes deposited on the surface of a microbe. Here, C_{3b} forms stable bonds with microbial products, such as endotoxins and zymosan of yeast cell wall, and is thus protected from degradation. C_{3b} which becomes deposited on host cell surface is prevented from binding stably by several regulatory proteins that are present on host cells but absent from microbes.

The microbe-bound C_{3b} forms a complex with factor B, this complex is the C₃ convertase of the alternative pathway. Factor D and properdin also help in the generation and stabilization of the C₃ convertase. This convertase breaks down more C₃ resulting in the attachment of more C_{3b} to the microbial surface. C_{3b} attached to the microbial surface activates the rest of the complement components in the same order as in the classical pathway to produce MAC.

N.B.: C₁, C₄ and C₂ are not activated in this pathway.

Antibodies have no role in the activation of complement via the alternative or lectin pathways. Therefore, these pathways are considered to be mechanisms of innate immunity, acting early before development of specific antibodies. On the other hand, the classical pathway requires the presence of antibodies and is, therefore, a part of the acquired immune response (Table 10).

Table (10): Comparison between the 3 complement pathways:

	Classical pathway	Lectin pathway	Alternative pathway
Type of immunity	Acquired (specific)	Innate (non-specific)	Innate (non-specific)
Initiation	Antigen-antibody complex	mannose Lectin binding to pathogen surface	Microbial components (e.g. endotoxin)
Role of antibodies	Needed for initiation (activation of C ₁)	Have no role	Have no role
Role of factor B, D and properdin	Have no role	Have no role	Have a role
Role of mannose-binding lectin	Has no role	Has a role	Has no role
The involved components	C _{1,4,2,3,5,6,7,8,9}	C _{4,2,3,5,6,7,8,9}	C _{3,5,6,7,8,9}
	slow	C ₁ is not	C _{4,2} is not
		Rapid	

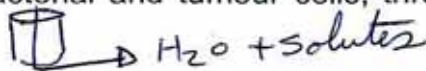
مفارقة



membrane attack complex

Functions of Complement

1. **Direct cytolysis:** Insertion of the MAC into the cell surface leads to killing of many cells, e.g. bacterial and tumour cells, through osmotic lysis (see Fig. 28, Chapter 16).



2. **Opsonization:** During complement activation, C_{3b} becomes deposited on the surface of the pathogen (antigen). Phagocytic cells recognize C_{3b} bound to the pathogen via their C_{3b} receptors. This facilitates the attachment and subsequent uptake and killing of the C_{3b} -coated pathogen by the phagocytic cell (see Fig. 28, Chapter 16).

3. **Immune complex clearance:** C_{3b} receptors are also found on RBCs. These recognize C_{3b} bound to soluble immune complexes. Erythrocytes bind the immune complexes via these receptors and transport them to organs rich in fixed phagocytes (e.g. liver and spleen). Using their own C_{3b} and Fc receptors, these phagocytes remove the immune complexes from the red cells. This helps clearance of soluble immune complexes from the circulation and prevents the development of immune complex diseases.

4. **Inflammatory response:** During complement activation, the by-products C_{3a} and C_{5a} are produced. These molecules, known as anaphylatoxins, have the following important biological activities:

- a- Degranulation of mast cells and basophils to release mediators of inflammation, e.g. histamine.
- b- Recruitment of phagocytic cells to the site of inflammation (chemotaxis) and stimulation of their phagocytic power and intracellular killing.

Regulation of the Complement System

Complement tends to undergo spontaneous activation, especially by the alternative pathway. The activated complement components can destroy any cell to which they bind. Host cells are protected from such damage by a series of complement-regulatory proteins, e.g. C_1 inhibitor binds to and inactivates C_1 preventing further cleavage of C_4 and C_2 . Some of these proteins are associated with the host cell surface, whereas others are plasma proteins. Deficiency of regulatory proteins results in excessive complement activation that causes inflammation and widespread cell death.

why they don't destroy cells they bind!

* why CSF & urine has no complement ptas?

@ urine $\Rightarrow$ excretion

@ CSF $\Rightarrow$ no need to get lysis & damage there bec. hardly get antigens there



A schematic representation of the complement pathways and functions is shown in Fig. (34).

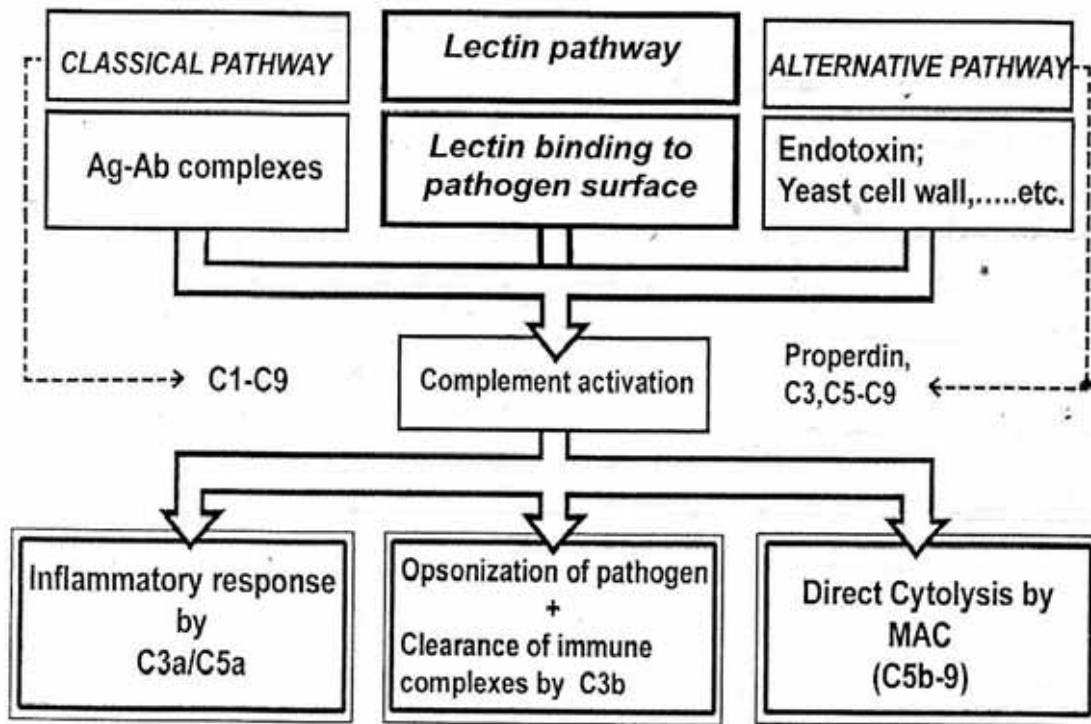


Fig. (34): Complement pathways and functions.

Chapter 18

IMMUNITY TO MICROBES

The principal physiologic function of the immune system is to protect the host against pathogenic microbes.

General features of immunity against microbes

1. Defence against microbes is mediated by both innate and specific immunity. Specific immunity enhances the protective mechanisms of innate immunity. Examples include the activation of macrophages by IFN- γ and activation of NK cells by IL-2 both of which are produced by T helper cells.
2. The innate immune response to microbes plays an important role in determining the nature of the specific immune response. Production of IL-12 by macrophages, for example, leads to development of Th1 cells and consequently a good cell-mediated immune response.
3. The immune system is capable of responding in different ways to different microbes, in order to combat these agents most effectively.
4. The survival of pathogenic microbes in a host depends on the ability of these microbes to evade or resist the body's immune mechanisms.
- * 5. During infections, tissue injury and disease may sometimes be caused by the host response to the microbe and its products rather than by the microbe itself.

I. Immunity to Extracellular Bacteria

Extracellular bacteria are capable of replicating outside host cells. Examples are the pyogenic (pus-forming) cocci as well as some enteric organisms.

A- Innate Immunity

- **Phagocytosis** by neutrophils, monocytes and tissue macrophages: Extracellular microbes are rapidly killed by the microbicidal mechanisms of phagocytes.
- Activation of complement by the alternative pathway: Complement can be activated by the peptidoglycan and lipopolysaccharide in the cell wall of bacteria. Complement activation leads to production of opsonins, recruitment and activation of phagocytes and lysis of bacteria.

B- Specific Immunity

Humoral immune response

This is the main protective specific immune response against extracellular bacteria. Antibodies perform several functions to eliminate extracellular bacteria:

1. Agglutination
2. Neutralization
3. Opsonization

Why Main role is to Antibody?

function
→ accessible to extracellular

P. 66

*Proof that acquired enhance Innate

→ Specific humoral is main

not for extracellular bacteria

bec.

extracellular bacteria

Microbiology

* Antibody function:

1. Opsonization.
2. Agglutination of bacteria, preventing spreading and facilitating phagocytosis.
3. Neutralization of bacterial toxins, preventing their binding to target cells.
4. Binding to pilin protein of the pili and inhibition of adhesion of bacteria to host cells.

Tip

5. Activation of complement by the classical pathway, with all its consequences.

• T cell response

As mentioned in Chapter 14, extracellular bacteria and their products are internalized by APCs and peptides from them are presented to T cells in association with MHC II molecules. Thus, the main T cell response is that of T helper cells. Their effector functions against extracellular bacteria are mediated by the cytokines they secrete and include:

- Stimulation of antibody production.
- Induction of local inflammation.
- Enhancement of phagocytic and microbicidal activities of macrophages.

II. Immunity to Intracellular Bacteria

A characteristic of intracellular bacteria is their ability to survive, and even replicate, within phagocytes, e.g. mycobacteria, which cause tuberculosis and leprosy.

A- Innate Immunity

- Pathogenic intracellular bacteria are resistant to degradation within phagocytes. Thus, innate immunity is usually ineffective in controlling infections caused by these organisms. This is also the reason why these organisms tend to cause chronic infections that last for years and may recur after apparent cure.

NK cells provide early defence against these microbes before specific immunity develops. They produce IFN- γ which activates macrophages to kill intracellular bacteria.

B- Specific Immunity

• Humoral immune response

Since intracellular bacteria are capable of finding a hiding place where they are inaccessible to circulating antibodies, humoral immunity does not play a role in their elimination.

• T cell response

Cell-mediated immunity (in the form of macrophage activation by Th1 cells) is the main protective immune response against intracellular bacteria. These bacteria induce the production of IL-12 by macrophages and IFN- γ by NK cells. Both these cytokines promote the development of Th1 cells. These, in turn, secrete IFN- γ which activates the macrophages to kill the bacteria that they harbour (see Fig. 21, Chapter 15).

Although the development of Th1 cells is very important for dealing with intracellular bacteria, sometimes this does not happen, and this has a great

Abstract of this
Representation
Immune
Complex
Clearance
Inflammatory
response

Th₂ → activate B cells

⇒ Cell mediated is main

Remark Class II MHC ⇒ cell mediated ⇒ Th

Antibody

G.R. doesn't play role

hide → macrophages

Can present in some patient but useless also
no accessible
hide in phagosome

Th₂ activation in Intracell.
is harmful

Th₀ → Th → IL-12 + IFN γ → Th₁

IFN γ
IL-2
TNF- α → Synergize

Why Hypersensitivity action of Th1 is good? = what if absent? Freely, you have received; freely give.

effect on the outcome of an infection with intracellular bacteria. A striking example is seen in the disease leprosy, which is caused by Mycobacterium leprae which requires macrophage activation by Th1 cells for combating it. Two forms of the disease are present:

In tuberculoid leprosy, Th1 cells are dominantly induced. The result is that there is intracellular digestion of the mycobacteria and few living bacteria are found in the tissues. Although the skin and peripheral nerves are damaged by the inflammatory responses associated with macrophage activation, the disease is under control and the patient survives. Benign

In lepromatous leprosy, Th2 cells are dominantly induced. The main response is humoral but this is useless since the antibodies cannot reach the intracellular bacteria. The organism grows abundantly in the macrophages causing much destruction and eventually death. Malignant

III. Immunity to Viruses

Viruses are obligate intracellular parasites which must replicate within cells, utilizing the nucleic acid and protein synthetic machinery of the host. They reside in the cytosol of the cell and, thus, peptides from them are presented on the cell surface in conjunction with MHC I molecules. Most viruses, after replication inside the cells, are liberated to infect other cells. On the other hand, certain viruses remain inside the cells they infect and replicate without causing cell lysis.

A- Innate Immunity

- NK cells:

Cytotoxic killing of virally infected cells by NK cells is one of the main mechanisms of immunity against viruses early in the course of infection.

- Type I interferon: $\Rightarrow$ because innate

Virally-infected cells produce type I interferon (IFN- α and IFN- β). These function in many ways to combat viral infections:

- They inhibit viral replication and induce an antiviral state.
- They activate NK cells.
- They increase the expression of MHC I molecules, allowing better presentation of viral peptides.

See details in Chapter 15.

B- Specific Immunity

- Humoral immune response

Specific antibodies are important in defence against viruses before they enter their target cells, and against viruses released from infected cells. This is because in these two situations, virus particles are accessible to antibodies.

Antibodies function against viruses in many ways:

1. Neutralizing antibodies bind to viruses and prevent viral attachment and entrance into host cells.
2. Opsonizing antibodies enhance phagocytosis.
3. Activation of complement by antibody may also promote phagocytosis or may possibly cause direct lysis of viruses with lipid envelopes.

$\rightarrow$ Leprosy = Hansen's disease

In general, secretory IgA is important in neutralizing viruses that enter through the mucosa, while circulating antibodies (IgM & IgG) are effective against viruses which pass through the blood stream before reaching their target cells. When comparing the antiviral action of type I IFN (innate) to antibodies (specific) it is evident that type I IFN acts early during viral infection, acts on viruses intracellularly and its action is not specific against a certain virus. On the other hand, antibodies act at a later stage against extracellular viruses and their action is specific.

کون کون کا ویس
Antibody
+
IFN

- T cell response
- Cytotoxic T cells:

IFN I	Antibody
• Innate non spec.	• acquired specific
• fast w/ high exposure	• delayed onset
• Intracellular	• Neutralize extracellular

virus

The main mechanism of specific immunity against established viral infections is killing of infected cells by Tc cells. This mechanism is effective against viruses before being released from the infected cell. It is particularly important against those viruses which tend to remain for long periods inside host cells without being released and which are, therefore, inaccessible to antibodies.

فائزات
کے
میں

- Helper T cells:

→ G.R. Why have role? IFN-γ

These contribute by secretion of cytokines. The Th1 cytokines IL-2 and IFN-γ are most important in this respect:

1. IL-2 promotes proliferation and activation of Tc cells and also activates NK cells.
2. IFN-γ activates NK cells.

* How T helper stimulated?
→ bec Internalization of virus in vacuole

IV. Immunity to Fungi

A- Innate Immunity

The neutrophil is the most important cell of the innate immune system in combating fungi. Neutrophils liberate fungicidal substances and phagocytose fungi as well. People with neutropenia are extremely sensitive to fungal infections.

- Macrophages can also combat fungal infections.

B- Specific Immunity

Most pathogenic fungi behave like intracellular bacteria and specific immunity to them is quite similar.

• Humoral immune response

Antibodies are often produced against fungi, but do not appear to be useful in protection.

• T cell response

Cell mediated immunity is the major defence mechanism against fungal infections. It acts in the same way as for combating intracellular bacterial infections. Similarly, the Th1 response is protective and the Th2 response is harmful to the host.

Th
Major

Intracellular bacteria &

* which Imp. more in Imm. to fungi
phagocytosis + fungicidal substance
neutrophil & macrophage?

Major

Th1	Th2
• Large dose antigen	• small dose antigen
	• resist degradation

* Person has Leukopenia more susceptible to fungal infection
* neutrophils Important.

Th1
Th2

±

Classification of Acquired (Specific) Immunity

Specific immunity against various infectious agents may be acquired either passively or actively (Table 11).



I. Active Acquired Immunity

طی + ذاک کو

Antigens of the microorganism must come in contact with cells of the immune system, resulting in specific stimulation of B and/or T cells. This form of immunity takes some time to develop (time needed to induce activation, proliferation and differentiation of lymphocytes). However, it lasts after the antigen is eliminated, due to development of immunological memory. There are two ways for acquiring active immunity:

A- Natural active immunity

This occurs following natural infections, whether clinical or sub-clinical. Some infections, such as measles, induce long-lasting immunity. Others, such as influenza, confer immunity lasting for a relatively shorter time.

B- Artificial active immunity

This occurs by deliberate administration of antigenic material from pathogens, a process called **vaccination**. The pathogens may be introduced whole, in a killed or attenuated (non-pathogenic) form, or parts of an organism may be used. Alternatively, toxoids may be used. Various vaccines are used routinely to protect people from infectious diseases, and this process is called **active immunization**.



II. Passive Acquired Immunity

سریج + مزاکرش کو

This involves transfer of ready-made antibodies and/or lymphocytes to an individual. The immune system has no active role in initiating an immune response. Immunity acquired here is immediate, but is temporary, remaining only as long as the transferred material remains active in the recipient's body. There are two ways for acquiring passive immunity:

A- Natural passive immunity

This is the transfer of maternal antibodies (IgG) to the foetus across the placenta, or passage of secretory IgA to the newborn in the colostrum. This protects the newborn against many diseases during the first six months of life.

B- Artificial passive immunity

- **Humoral immunity** can be artificially transferred to an individual by transfer of antibodies. This is called **passive immunization**. Examples include:
 - Administration of **antitoxic serum** for treatment or prophylaxis against infections caused by bacteria that produce exotoxins, as in diphtheria and tetanus. (Antitoxic serum is obtained by repeated injection of human volunteers or animals with a toxoid, provoking production of antibodies against it, so that their serum is rich in that specific antitoxin and can be administered to another individual).



- Administration of **gamma globulin** to an immunodeficient person. (Gamma globulin is pooled serum obtained from normal adults and contains a mixture of many protective antibodies).
- Administration of **convalescent serum** to exposed people to protect from diseases such as infectious hepatitis. (Convalescent serum is serum obtained from a person convalescing from a certain infectious disease and is, therefore, rich in specific antibodies).
- **Cell-mediated immunity** can be transferred by transfer of lymphocytes. However, such cell transfer must be between genetically identical individuals to avoid rejection reactions. It is, therefore, not suitable for clinical use, but is used experimentally in genetically identical animals.

G.R. Why transfer is not practical?

Table (11): Comparison between active and passive immunity:

	Active	Passive
Role of immune system	Important role	No role
Mechanism	<u>Stimulation of B/T cells</u>	<u>Transfer of ready-made antibodies/lymphocytes</u>
Onset of protection	Delayed	Immediate
Duration of protection	Longer	Shorter
Development of memory	Yes	No
Examples	-Natural infections -Active immunization with <u>vaccines</u>	-Maternally-acquired antibodies -Passive immunization with <u>antitoxic serum</u>

Last after antigen eliminated

remain as long as material transferred active

diphtheria + tetanus



Chapter 19

TUMOUR IMMUNOLOGY

It has been suggested that tumours look like an allograft in relation to rejection mechanisms by the body. Tumour rejection mechanism represents a means by which the body cells could be kept under immuno-surveillance. Immuno-surveillance is the ability of the immune system to prevent the development of most tumours through early recognition and destruction of tumour cells. For this to operate, tumour cells must develop surface membrane molecules which might be antigenically new (**tumour antigens**) and can be recognized by the immune system.

Tumour Antigens

TSAs
TAAs

Two groups of tumour antigens have been described:

Tumour-specific antigens (TSAs) are antigens that are expressed on tumour cells but not on normal cells and might, therefore, induce an active immune response.

Tumour-associated antigens (TAAs) are antigens that are relatively restricted to tumour cells but may also be present on normal tissue and, therefore, may not be able to stimulate an effective response.

Any tumour antigen, from either group, that contributes to tumour rejection is referred to as tumour-associated transplantation antigen (TATA).

Origin of tumour antigens

- 1. Oncofoetal antigens:** These are present during normal foetal development but are lost during adult life. They may reappear with the development of tumours. Examples are alpha foeto-proteins (AFP) in hepatoma and carcinoembryonic antigen (CEA) in colon carcinomas.
- 2. Tumour associated transplantation antigens (TATA) on virally-induced tumours:** These are virally derived peptides associated with surface MHC on the tumour cell. They behave as powerful transplantation antigens. They are present mainly on tumours produced by oncogenic viruses e.g. Epstein-Barr virus (EBV) in lymphomas, and hepatitis B virus in hepatic carcinoma.
- 3. Tissue-specific differentiation antigens:** A tumour arising from a particular tissue may express normal differentiation antigens specific for that tissue, e.g. prostate-specific antigen.

Evidence for Immune Response (Immuno-Surveillance) to Tumours

- Immunosuppressed patients (e.g. AIDS patients) develop tumours frequently.
- Very young and very old people have an increased incidence of tumours. These members of the population often have less effective immune response.



3. Antibodies and T lymphocytes against tumour antigens have been detected in patients with tumours.
4. Animals can be specifically immunized against various types of tumours.

Effector Mechanisms Involved in Tumour Immunology

Components of the immune system (both innate and specific; humoral and cellular) can affect the growth and progression of a tumour through different mechanisms:

I. T Cells:

These are the main cells responsible for anti-tumour immunity:

- Main* →
1. **Cytotoxic T cells** recognize antigens in association with class I MHC molecules on tumour cells and kill them. These cells are thought to play the most important role in recognition of tumour cells and their destruction.
 2. **Helper T cells** are activated by shed tumour antigens which become internalized and presented on the surface of APC in association with MHCII. They secrete cytokines which activate Tc cells, macrophages, NK cells and B cells. Th cells also produce TNF which is directly toxic to tumour cells.

II. B Cells:

1. B cells (as APCs) may play an important role in processing and presenting tumour antigens to Th cells.
2. They produce tumour-specific antibodies, which may cause tumour cell lysis by:
 - a. Fixing complement to the tumour cell membrane, resulting in the formation of membrane attack complex and consequent lysis of tumour cells.
 - b. Antibody-dependent cellular cytotoxicity (ADCC) mediated by NK cells and possibly other cells such as macrophages.

III. Natural Killer Cells

1. NK cells recognize tumour cells and kill them.
2. NK cells also kill tumour cells by ADCC.

N.B.: The activity of NK cells is enhanced by IL-2 and INF- γ produced by Th1 cells.

IV. Monocytes/Macrophages

Monocytes/macrophages play an important role through:

1. Antigen presentation and cytokine secretion.
2. ADCC.
3. Direct cytotoxicity of the tumour cells by releasing TNF- α , nitric oxide and hydrogen peroxide.

Tumour Evasion (Escape) of the Immune Response

A number of mechanisms have been suggested for the escape of tumours from immuno-surveillance:

1. Host may be immunocompromised.
2. Factors related to tumour antigens:
 - Some tumours lack antigens that can stimulate the immune response.
 - Some tumour antigens cannot be processed and presented with MHC.
 - Amount of antigen may be too small to stimulate the immune system.
 - Some tumours may shed their antigens which block antibodies and T cells from reacting with the tumour cells.
3. Tumours localized at sites inaccessible to the immune system e.g. **CNS**.
4. Certain tumours (virally-induced) lack or are poor in expression of MHC I molecules.
5. Some viruses block expression of co-stimulatory molecules (e.g. B7) by antigen-presenting cells.
6. Blocking antibodies: Non-cytolytic (non-complement fixing) antibodies in the serum may bind to the tumour antigens, making them inaccessible to complement fixing antibodies. This prevents complement mediated lysis of tumour cells.
7. Fibrin coating leads to masking of tumour antigens.
8. Some tumours may secrete substances, such as TGF- β , that suppress the immune response directly.

Tumour Immunodiagnostics (Tumour Markers)

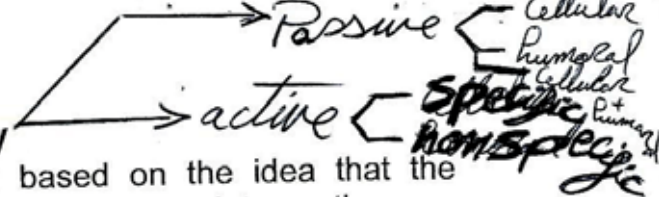
Tumour antigens can be very useful **tumour markers** in the diagnosis and follow-up of various tumours. An ideal tumour marker is: 1) released only from tumour tissue, 2) specific for a given tumour type, 3) detectable early upon tumour formation, 4) its concentration in the blood is proportional to the tumour mass, and 5) present in all patients with the tumour. Most tumour markers are antigenic, circulating in the blood, and can be detected by immunoassays. Although useful in monitoring patients for tumour recurrence after therapy, no tumour marker has definite specificity or sensitivity for application in early diagnosis. Examples of tumour markers are:

1. **Carcinoembryonic antigen (CEA)**: in colon carcinomas.
2. **α -Foetoprotein (AFP)**: in primary hepatoma.
3. **β -Subunit of human chorionic gonadotrophin (β -HCG)**: in choriocarcinoma.
4. **Prostate-specific antigen (PSA)**: in cancer prostate.
5. **CA 125**: in ovarian cancer.
6. **CA 19-9**: in colonic and pancreatic tumours.
7. **CA 15-3**: in breast cancer.
8. **Bence-Jones proteins**: in myeloma.
9. **Pancarcinoma antigen (TAG-72)**: is used to localize occult tumour secondaries by using radiolabelled monoclonal antibody.

*1 Mention 1 example for each of 2

Approaches to Tumour Immunotherapy

Attempts at using immunotherapy have been based on the idea that the immune system can eradicate some forms of existing tumours. Intervention may be passive or active, specific or non-specific.



I. Passive Cellular Immunotherapy

Passive cellular immunotherapy is a term used when activated cells are directly infused into a patient:

1. **Therapy with lymphokine-activated killer (LAK) cells:** The patient's lymphocytes (including NK cells) are cultured *in vitro* with IL-2 then reinfused.
2. **Therapy with T cells:** In this approach, lymphocytes that have infiltrated tumours are extracted from tumour biopsies, stimulated *in vitro* using IL-2 and tumour antigen and then reinfused.

II. Passive Humoral Immunotherapy

Monoclonal antibodies directed against tumour antigens may be used either alone or coupled to radioisotopes, cytotoxic drugs, toxins (e.g. diphtheria toxin) or cytokines. Coupling has the advantage of delivering high doses of radioactivity or cytotoxic drugs directly to the site of the tumour (**magic bullet**).

III. Passive Cellular and Humoral (Combined) Immunotherapy

This is a new approach, based on the development of bispecific (bifunctional) antibodies, which links one antibody reacting with the tumour cell to a second antibody reacting with a cytotoxic effector cell, targeting the latter directly to the tumour.

IV. Active Specific Immunotherapy

1. **Allogeneic tumour cells:** These are tumour cells taken from other patients, irradiated and then injected with BCG vaccine or other adjuvants.
2. **Tumour antigen vaccines** are among the most promising approaches in cancer immunotherapy. Cellular immunity to specific, well-defined antigens can be induced by using short synthetic peptides.

N.B.: Active immunization against oncogenic viruses can prevent the development of certain tumours. The best example is the successful mass immunization against hepatitis B virus which is already decreasing the incidence of primary hepatoma in endemic areas.

V. Nonspecific Immunotherapy

1. **Interferons (IFNs)** have anti-tumour activity in leukaemia and AIDS-associated Kaposi's sarcoma.
2. **Bacterial adjuvants** e.g. BCG, or killed suspensions of *Corynebacterium parvum* have been used with or without tumour antigens, to treat a wide variety of cancers, usually along with intensive chemotherapy or radiotherapy.

Magic Bullet

Chapter 20

TISSUE INJURY IN IMMUNOLOGICAL REACTIONS (IMMUNOPATHOLOGY)

In the previous chapters, we discussed how immunological mechanisms can operate to the benefit of the host in overcoming microbial infections and in protecting from tumours. It is the fighting army for the host to preserve his integrity. However, there is no war without victims. So, it might be expected that some forms of tissue damage may arise from exaggerated or inappropriate immunological responses.

The mechanisms by which immunological tissue damage occurs are classified into 4 categories:

1. Anaphylactic reaction.
2. Cytotoxic (cytolytic) reaction.
3. Immune-complex-mediated reaction.
4. Cell-mediated reaction.

These reactions may occur in cases of hypersensitivity, auto-immune disease or graft rejection.

① HYPERSENSITIVITY

Occur in 2nd exposure

Hypersensitivity reactions are inappropriate immune responses to certain antigens causing tissue damage. Typically, hypersensitivity reactions occur following re-exposure of an individual to the antigen; the first contact induces an immune response (sensitization), while subsequent exposure produces a reaction that leads to tissue damage.

* 1st exposure → immune response (sensitization)
* 2nd " → tissue damage

Hypersensitivity reactions have been classified into 4 types that correspond to the 4 mechanisms of tissue damage.

TYPE I: IMMEDIATE (ANAPHYLACTIC) HYPERSENSITIVITY REACTIONS

Type I hypersensitivity reactions are exaggerated immune responses to harmless environmental antigens (allergens). They occur almost exclusively in hypersensitivity (not during autoimmune disease or graft rejection). They are mediated by IgE.

Mechanism: (Fig. 35)

A- Sensitization

- In normal individuals, antigenic stimulation results in the production of extremely low levels of IgE. However, certain individuals have a genetic predisposition to allergy. They respond to certain antigens (allergens) by forming large amounts of IgE.

Th2 → ↑ IL-4 → class switch from IgM → IgE → IgE ↑ + bind Fc on mast Basophil → sensitization

Cross link by allergen

histamine → early phase
Leukotrienes
prostaglandin → late phase

early phase
late phase

- The production of IgE is under the control of Th2 cells. These cells produce ↑ IL-4, which is the critical stimulus for inducing class switching from IgM to IgE, resulting in the production of large amounts of allergen-specific IgE.
- The IgE molecules bind to Fc receptors on mast cells (and basophils). Thus, first exposure to an allergen results in sensitization of the mast cells (and basophils).

B- Degranulation of mast cells

Upon subsequent exposure to the same allergen, the IgE molecules on the sensitized mast cells become cross-linked by the allergen. This induces degranulation of mast cells and the release of 2 kinds of mediators:

a- Preformed mediators: Histamine and platelet activating factor (PAF) are the mediators of symptoms seen during the early phase, which occurs within 15-20 minutes of exposure to the allergen.

b- Newly formed mediators: The leukotrienes and prostaglandins, which take several hours to be synthesized, cause the symptoms seen during the late phase. The late phase typically occurs 5-6 hours after allergen contact. The symptoms of the late phase are the same as those of the early phase but persist longer.

This late phase involves the recruitment of other effector cells, notably Th2 lymphocytes, eosinophils and basophils which contribute significantly to the inflammatory response and tissue damage.

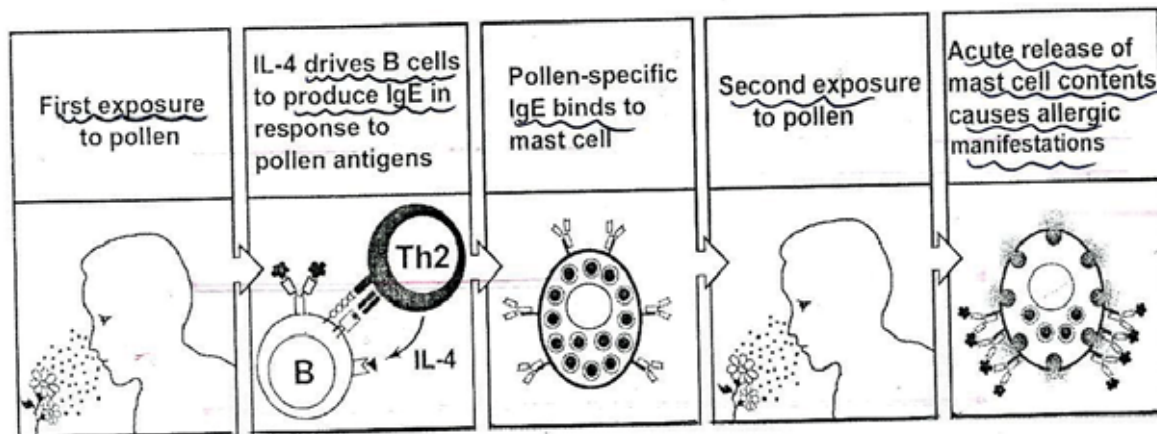


Fig. (35): Mechanism of type I hypersensitivity reactions.

Clinical Forms

1. Systemic anaphylaxis:

This is the most severe form of type I hypersensitivity, in which severe bronchoconstriction and hypotension (shock) can be life-threatening.

Anaphylaxis may occur due to previous sensitization by foreign antigens, e.g. antitoxic serum prepared in animals for treatment of tetanus and diphtheria. It also occurs with penicillin and many other drugs that act as haptens and attach to body proteins.

systemic (ingestant) → Bronchoconstriction
hypotension (shock)

Local (Atopy) → in one organ

allergen → Inhalant
Contactant
Ingestant

↑ Capillary permeability
↑ mucous secretion

2. Localized anaphylaxis (Atopy)

In this form, the symptoms are localized in one organ or system. The allergens to which the individual is sensitized are widely distributed in nature. They are classified as inhalants, e.g. pollens, house dust, moulds, contactants like wool, animal fur, nylon and ingestants including many food stuffs. The symptoms vary widely from one individual to another depending on the site of antigen-antibody reaction. Thus, it may take the form of bronchial asthma, urticaria, conjunctivitis, gastrointestinal symptoms or allergic rhinitis.

There is hereditary tendency in atopy. Atopic individuals respond to certain allergens by producing larger amounts of IgE than normal individuals.

Diagnosis

1. Detection of the antigen to which the individual is sensitive by skin test in which extracts of various antigens are introduced into the skin of the patient.
★ Positive cases show a wheal and flare reaction that appears within 15-25 min. at the site of the antigen to which the individual is allergic.
2. Measurement of levels of total IgE and IgE specific for a particular allergen.

Therapeutic Measures

I. Management of anaphylactic shock

Anaphylactic shock is an emergency which must be dealt with immediately by administration of adrenaline, corticosteroids together with oxygen inhalation.

II. Management of atopy

1. Avoidance of the responsible allergen.
2. Desensitization involves injecting the patient, over time, with gradually increasing doses of the responsible allergen. This form of immunotherapy aims at shifting the immune response from Th2 to Th1 response which down regulates IgE production.
3. Drugs that block the release of the mediators or counteract their effects, e.g. antihistaminics, corticosteroids and anti-leukotrienes.

TYPE II: CYTOLYTIC (CYTOTOXIC) HYPERSENSITIVITY REACTIONS

This type of hypersensitivity is mediated by IgG or IgM antibodies reacting with cell-bound antigen (surface antigen). This antigen may be originally present on the cell membrane or an antigen (or hapten) that became attached to it. This cell may then be destroyed by one of the following destructive processes (Fig. 36):

- a. Lysis of target cells via activation of complement.
- b. Opsonization of target cells with or without complement activation.
- c. Lysis of target cells by antibody-dependent cell-mediated cytotoxicity (ADCC) through the action of natural killer (NK) cells, polymorphs or macrophages.

وكان الراجح مع سيدنا محمد ﷺ فكان رجلاً ناهياً

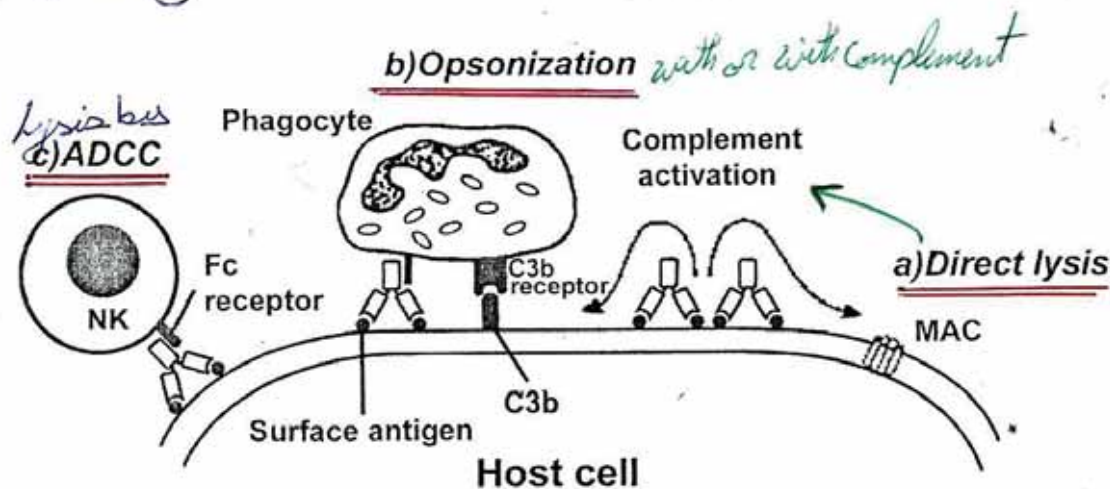


Fig. (36): Mechanism of type II hypersensitivity reactions.

Examples of Cytolytic (Cytotoxic) Reactions

1. Blood group (ABO or Rh) incompatibility.
2. Autoimmune diseases, e.g. autoantibodies to an individual's own red blood cells are present in autoimmune haemolytic anaemia. Autoantibodies to other tissues, e.g. kidney and skin may be produced in certain diseases and lead to tissue damage of the respective organs.
3. Drug reaction: A drug may become attached to a cell surface. Antibodies to the drug reacting at the cell surface lead to destruction of the cell in presence of complement.
4. Graft rejection.

Blood gp Incompat.
Autoimmune disease
drug reaction
graft rejection

TYPE III: IMMUNE-COMPLEX HYPERSENSITIVITY REACTIONS

★ When antibodies combine with their specific antigen, immune complexes are formed. Normally, they are effectively removed by the reticuloendothelial system (macrophages). Occasionally, small soluble immune complexes are formed, especially when there is antigen excess. These complexes, being very small, may escape phagocytosis and become deposited in tissues (especially in joints and kidneys) leading to tissue damage.

soluble antigen
bec. Immune Complex
circulate then ppt.

Mechanism of Tissue Damage: (Fig. 37)

1. The first step is the formation of soluble immune complexes that are formed of antigen and IgG or IgM.
2. These immune complexes penetrate the endothelium of blood vessel walls and become deposited on the vascular basement membrane.
3. Complement is activated and C_{3a} and C_{5a} (anaphylatoxins) are released:
 - a. These anaphylatoxins react with receptors on mast cells and basophils, causing release of vasoactive amines (e.g. histamine), which increase vascular permeability.

Zone phenomenon

What is role of C_{3a} & C_{5a} ?

in Type III? Anaphylatoxins + الشرح

- b. C_{5a} is also chemotactic for neutrophils which infiltrate the area. In an attempt to engulf the immune complexes, these phagocytic cells degranulate and release lysosomal enzymes that destroy the basement membrane.
4. Platelets are aggregated with two consequences; they release vasoactive amines and form microthrombi which cause local ischaemia and further tissue damage.

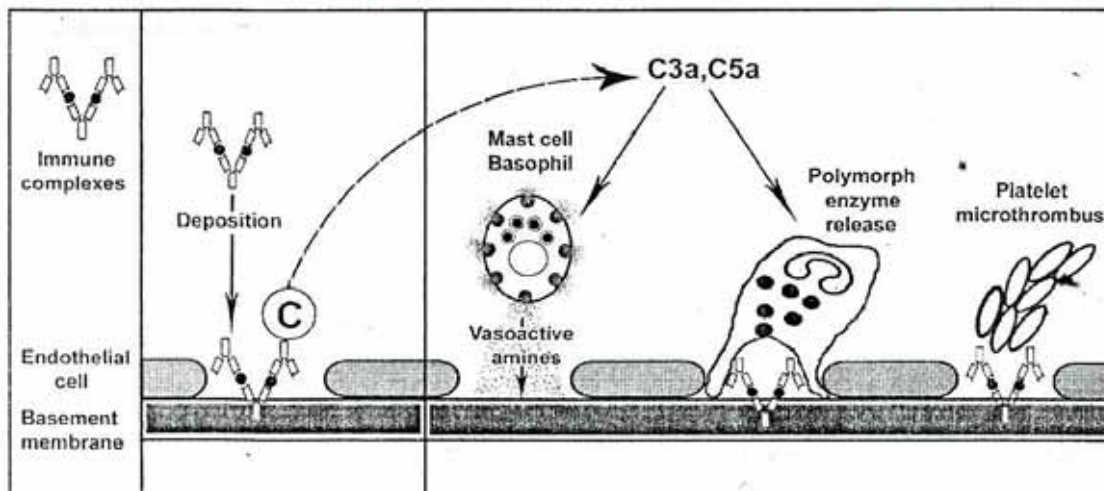


Fig. (37): Mechanism of type III hypersensitivity reactions.

Examples of Immune-Complex-Mediated Reactions

1. Serum Sickness

Systemic - Generalized

This syndrome is considered to be a systemic immune-complex disease. It occurs following injection of large amount of foreign serum (e.g. horse antitetanic or antidiphtheritic serum) or drugs (e.g. penicillin) into humans. The antigen is slowly cleared from the circulation, while antibody production begins. These antibodies react with remnants of antigens still present. Immune complexes are formed, which may become deposited at various sites causing the manifestations of the disease. Typical serum sickness results in fever, urticaria, arthralgia, lymphadenopathy, and splenomegaly, which develop a few days to 2 weeks after injection of the foreign serum or drug.

2. Arthus reaction

Local - at site of s.c. Injection

This is a form of local immune complex disease due to repeated subcutaneous injection of low dose of a foreign antigen, e.g. insulin and rabies vaccine. The reaction occurs at the site of antigen injection. Immune complexes are deposited in the walls of blood vessels, complement is activated, and the previously mentioned inflammatory response is initiated resulting in local erythema, oedema and necrosis.

3. Post-streptococcal glomerulonephritis.

4. Viral infections: e.g. hepatitis B.

5. Autoimmune diseases: e.g. rheumatoid arthritis and systemic lupus erythematosus (SLE).

6. Hypersensitivity pneumonitis: Some inhaled antigens, e.g. dust, pollen and fungal spores provoke IgG rather than IgE antibody responses. Immune complexes are formed in the alveolar wall leading to pneumonitis.

Serum sickness

Arthus reaction

Or. 0.5

Therapeutic Measures

1. Reduction of inflammation by means of antihistaminics and corticosteroids.
2. Suppression of the immune response by means of corticosteroids and immunosuppressive drugs.
3. Removal of offending complexes via plasmapheresis (exchanging the patient's plasma with normal plasma, thereby removing the immune complexes).

TYPE IV: CELL MEDIATED (DELAYED) HYPERSENSITIVITY REACTIONS

It is an exaggerated cell mediated immune response that damages host cells. The main cell involved is the activated T-lymphocyte (Th1). Antibody and histamine play no role in this type. The response is delayed (starts hours or days after contact with the antigen).

Mechanism: (Fig. 38)

Th1 cells recognize antigens and release pro-inflammatory cytokines. These act on the vascular endothelium leading to increase vascular permeability and leakage of fluid into the tissues. They also attract and activate monocytes, macrophages as well as more T cells. These inflammatory reactions lead to local tissue damage. Cytotoxic T cells may also play a role.

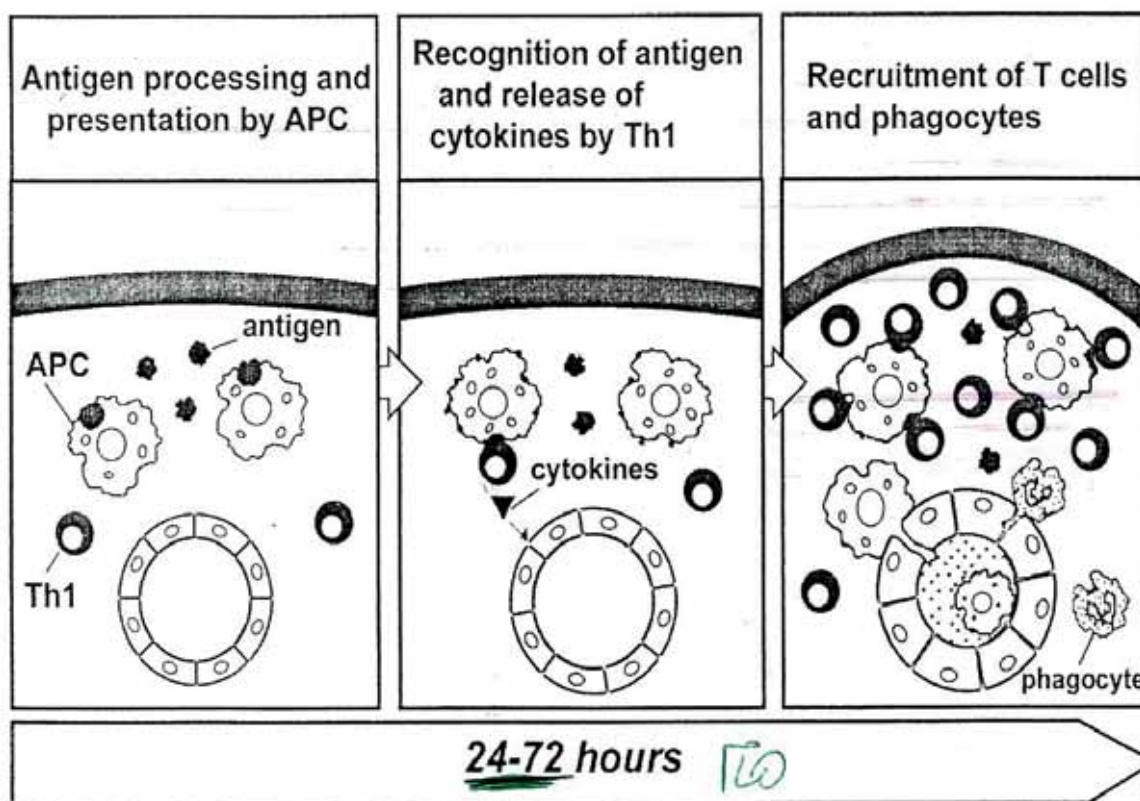


Fig. (38): Mechanism of type IV hypersensitivity reactions.

Examples of Cell-Mediated Reactions

1. **The tuberculin skin test** reaction is the classic clinical example of delayed hypersensitivity:

- A small amount of the antigen, purified protein derivative (PPD) of tubercle bacilli, is injected intradermally into a person.
- In a person previously exposed to tubercle bacilli (sensitized), a positive reaction occurs as an indurated area, 48-72 hours after injection. This induration is due to accumulation of macrophages and lymphocytes.

2. Contact dermatitis

The manifestations of cell mediated hypersensitivity occur after sensitization with chemicals, plant materials, topically applied drugs and some cosmetics. In all cases, the small molecules acting as haptens enter the skin, attach to body proteins, which are taken up by APCs in the skin (Langerhan's cells) to be presented to T cells. Upon a later skin contact with the offending agent, the sensitized person develops erythema, itching, vesicles, or eczema of the skin within 12-48 hours.

* 3. Granuloma formation

→ to isolate & kill die

Cell-mediated hypersensitivity reactions are seen in a number of chronic infectious diseases, especially those caused by intracellular bacteria, e.g. mycobacteria. When intracellular bacteria resist the microbicidal effects of the activated macrophages, the antigens persist and give rise to a chronic antigenic stimulus. Thus, continual release of cytokines from Th1 cells results in the accumulation of large numbers of activated macrophages. The result is the formation of a granuloma. This consists of a central core of infected macrophages. The core may include multinucleated giant cells, which are fused macrophages, surrounded by large macrophages called epithelioid cells. The central core is surrounded mainly by Th cells. Granuloma formation is the body's attempt to isolate pathogens that resist destruction. The centre of the granuloma become isolated and the cells die and because the dead tissue in the centre resembles cheese this process is called caseation necrosis. Thus, the cell-mediated immune reaction, in a situation where it does not eliminate the pathogen, can lead to tissue destruction. However, its absence can lead to death from disseminated infection. This is typically seen in AIDS patients with mycobacterial infection, since the number of Th cells in these patients is very low.

4. Autoimmune diseases.

5. Graft rejection.

TRANSPLANTATION IMMUNOLOGY

The Major Histocompatibility Complex (MHC)

MHC antigens are a group of molecules expressed on cell surface membranes. They are also called HLA because they were first discovered on the surface of human leucocytes. They may act as immunogens when introduced to a genetically distinct individual. These molecules are products of a group of genes known as the major histocompatibility complex (MHC) which are found on the short arm of chromosome 6. MHC genes are divided into three major classes; class I, class II and class III (Fig. 39).

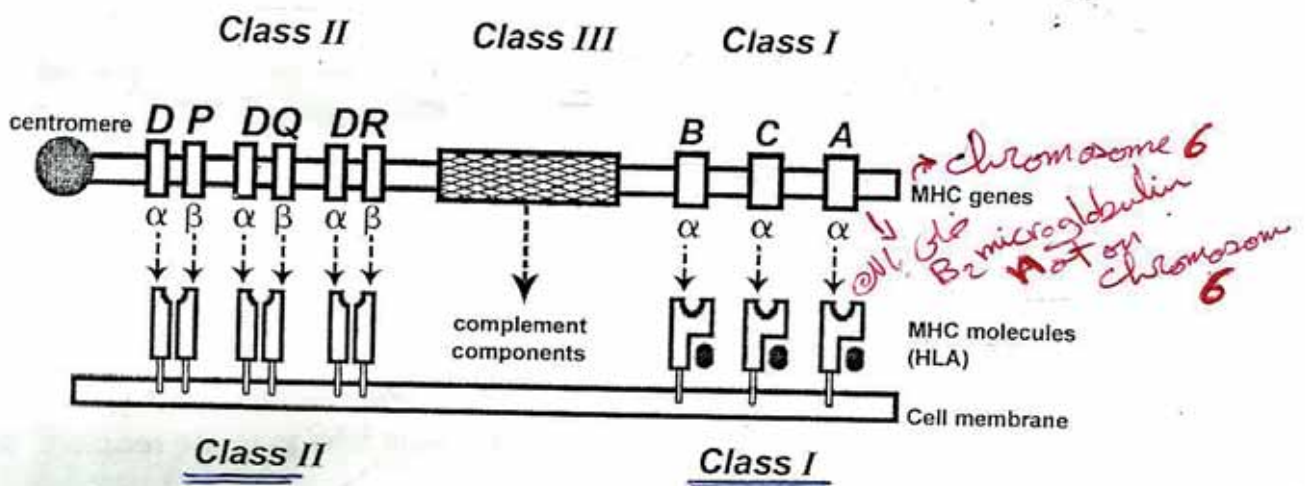


Fig. (39): The major histocompatibility complex (MHC)

MHC molecules are co-dominantly expressed from both maternal and paternal chromosomes. There are three class I loci (HLA-A, B and C). Each locus is highly polymorphic i.e. a single HLA locus contains one of many possible alleles. The various possible alleles are given consecutive numbers, e.g. HLA-A1, HLA-A2, etc. Thus, most individuals will have genes for six different class I molecules, all of which will present at the cell surface. Class I MHC molecules are found on the surface of all nucleated cells. Each molecule is composed of two polypeptide chains: heavy or α chain and β 2-microglobulin chain. The MHC genes code for the heavy chains (α chains), while β 2-microglobulin chain is coded for elsewhere in the genome.

Class II molecules are encoded by three principal loci (HLA-DP, DQ and DR), which also show polymorphism. Class II MHC molecules have a much more limited cellular distribution. They are mainly found on the surface of the professional antigen presenting cells (APCs). It is a dimer composed of two glycoprotein chains: α chain and β chain. MHC class II genes code for both α and β chains.

* Alleles: variants of a single genetic locus.

The class III genes code for a number of complement components and are grouped together in a region between HLA D and HLA B.

Significance of MHC

1. Antigen presentation to naïve T cells, leading to their activation.
 - CD8 • MHC I molecules enable effector Tc cells to recognize target cells.
 - CD4 • MHC II molecules on macrophages and B cells enable effector Th cells to recognize and help them.
2. Graft rejection is a consequence of mismatching of donor and recipient MHC molecules.
3. Certain MHC alleles affect the susceptibility of an individual to some inflammatory or autoimmune diseases (e.g. ankylosing spondylitis and B27 rheumatoid arthritis and DR4, and systemic lupus erythematosus and DR3).
4. They are used in forensic medicine, e.g. disputed paternity.

The Minor Histocompatibility Complex

This is a group of genes which code for antigens that cause weaker rejection responses than MHC antigens.

TRANSPLANTATION

Types of Grafts

1. **Autografts:** are grafts from one part of the body to another. They are not foreign and, therefore, do not elicit rejection.
2. **Isografts:** are grafts between genetically identical individuals, such as identical twins. As they do not express antigens foreign to the recipient, they are not rejected.
3. **Allografts:** are the common clinical transplants, where one person donates an organ to a genetically different individual of the same species. In this case, the cells of the allograft express antigens which are recognised as foreign by the recipient.
4. **Xenografts:** represent the maximal genetic disparity between members of different species and are generally rapidly rejected.

Antigens Responsible for Immune Rejection

1. Blood group antigens of the ABO system.
2. Major histocompatibility complex (MHC) antigens.
3. Minor histocompatibility complex antigens.

Types and Mechanisms of Allograft Rejection

1. **Acute Rejection** *only Type IV Hypersensitivity*
It is the most common type of rejection. It is a type IV (cell mediated) delayed hypersensitivity reaction. It takes days or weeks to develop because of the time taken for T cell activation, proliferation and migration into the donor graft. It takes place when there is HLA incompatibility.

Both Tc and Th lymphocytes are involved in the process of rejection:

- Tc cells cause direct destruction of transplanted cells.



- Th cells release a number of cytokines which lead to activation of the immune response. IL-2 is the most important cytokine in cellular rejection and is required for activation of Tc cells. TNF- α and IFN- γ act as macrophage activators. They also up-regulate the expression of adhesion molecules on vascular endothelium, which is important for the adhesion of blood-borne leucocytes to the walls of blood vessels.

II. Hyperacute Rejection

Type I Hypersensitivity & Type III

It is the most dramatic form of rejection. It takes place within minutes of transplantation, and is caused by preformed anti-donor antibodies binding to either ABO blood group or HLA class I antigens on the graft. Such antibodies attach to the endothelial cells of the donor organ, fix complement and cause damage to the vascular endothelium, resulting in haemorrhage, platelet aggregation within the vessels, graft thrombosis, lytic damage to cells of the transplant, and the release of the pro-inflammatory complement components

C_{3a} and C_{5a}.

III. Chronic or Late Rejection

Type II & III & IV Hypersensitivity

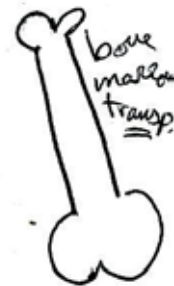
It takes place months or years after transplantation, depending on the genetic disparity between donor and recipient and the success of immunosuppressive therapy. This may be due to low-grade cell-mediated rejection or the deposition of antibodies or antigen-antibody complexes in grafted tissue.

It has been noticed that chronic rejection is more prominent in patients who have chronic viral infections, especially with cytomegalovirus or pre-existing autoimmune disease.

IV Graft-Versus-Host Disease (GVHD)

This condition occurs when the graft reacts against the recipient's tissues, instead of the recipient reacting against the graft. It can happen following bone-marrow transplantation in which immunologically competent T cells are transplanted into a genetically non-matching immunosuppressed recipient.

Careful typing, use of immunosuppressive drugs and removal of mature T cells from the donor stem cells, reduce the risk of GVHD.



Prevention of Rejection

1. Selection of Donor:

a- ABO matching: It is the first step in choosing the donor to avoid hyperacute rejection by preformed antibodies against the ABO blood group system.

b- Histocompatibility testing: A perfectly matched donor and recipient would be genetically identical individuals (monozygotic twins). However, this situation is rare, and in all other cases there will be major and/or minor histocompatibility differences between donor and recipient.

Since leucocytes carry all known HLA antigens, they can be used for tissue typing to identify HLA of both donor and recipient and to test for compatibility between them:

i. Tissue typing

• Serological HLA typing (Lymphocytotoxicity test):

A purified suspension of lymphocytes is mixed with antisera against the different HLA antigens in the presence of complement. If the lymphocytes carry the appropriate antigen, they will combine with the antibodies in the serum and activate the complement, leading to cell death. Trypan blue dye is added to differentiate between dead and living cells.

Example

B27	Anti	Antibody	Complement	Cell damaged
positive +	HLA-B27 →	reacts →	fixed →	and dye enters
cell				

B27	Anti	Antibody	Complement	Cell remains
positive +	HLA-B8 →	does not →	not fixed →	viable and
cell		react		unstained

• Molecular HLA typing:

Recently, sensitive and accurate typing has been achieved using PCR to identify HLA genes of donors and recipients.

ii. Compatibility testing by the mixed lymphocyte reaction (MLR):

It is used to test the responsiveness of the recipient lymphocytes to antigens expressed on donor cells. Donor and recipient cells are incubated together. Recognition of foreign HLA antigens on donor cells leads to proliferation of the recipient's T cells. The amount of proliferation can be measured by the extent of uptake of radioactive thymidine. The greater the extent of proliferation, the higher the disparity between donor and recipient. Low responses are associated with excellent transplant survival.

In case of bone marrow transplantation, it is important to assess response of donor lymphocytes to recipient antigens, in order to avoid GVHD.

2. Immunosuppressive Therapy

This is required to prevent and/or treat graft rejection and GVHD. It may be used as a maintenance therapy or in treating episodes of rejection. These drugs carry the risk of infection as a consequence of immunosuppression.

- ① Corticosteroids: They are potent anti-inflammatory agents.
- ② Inhibitors of cytokine production: Cyclosporine and tacrolimus inhibit T cell cytokine production (mainly IL-2 and IFN- γ).
- ③ Anti-proliferative drugs: Azathioprine and methotrexate inhibit DNA production, thus, preventing lymphocyte proliferation.
- ④ Monoclonal antibodies: For example, monoclonal anti-CD3 antibodies block the function of T cells.

3. Antigen Specific-Induced Tolerance

Induction of low-zone or high-zone tolerance to graft antigens without affecting protective immune responses is still under trial.

TOLERANCE AND AUTOIMMUNITY

TOLERANCE

Tolerance is the absence of specific immune response against some antigens in an otherwise fully immunocompetent person. It includes autotolerance and acquired (induced) tolerance.

I. Autotolerance

تعريف التولرجنس
Tolerance

It is a tolerance to self antigens that is acquired early in life, probably in utero. Failure of autotolerance may result in autoimmune disease.

Mechanism of Autotolerance

1. Central tolerance

It is proposed that during development in the primary lymphoid organs, B and T lymphocytes go through a phase in which contact with antigen leads to their death or permanent inactivation. Such antigens are most likely to be self-antigens. The elimination of immature self-reactive lymphocytes during their maturation is called negative selection (clonal deletion).

2. Peripheral tolerance

- Sometimes elimination of self-reactive cells in the primary lymphoid organs is not complete. This is probably because not all self-antigens are expressed in the primary lymphoid organs; therefore, the developing lymphocytes do not encounter them.
- Self-reactive B cells may reach maturity and migrate to the secondary lymphoid organs. However, they are controlled by lack of T cell help (Th cells specific to this particular antigen have been deleted).
- As with B cells, not all self-reactive T cells are deleted in the thymus. Such T cells are normally rendered unable to respond by various mechanisms which prevent their stimulation.

ال B موجود
ال T
عشره موجود

ولم يحان T موجود
unable to
respond
فيسبب
ال

II. Acquired (Induced) Tolerance

This is the induction of tolerance to an antigen at any time during life. It can be used as an approach for the therapy of autoimmune diseases, allergic conditions and graft rejection. The antigen is given in a certain form and under certain circumstances which favour the development of tolerance. Such an antigen is called a tolerogen.

Factors influencing the induction of tolerance

- High doses of antigen usually tolerize B-cells while minute doses given repeatedly tolerize T-cells. A moderate dose of the same antigen might be immunogenic.
- Protein antigens are more tolerogenic when in a soluble form than in aggregated or particulate form.

3. The tolerogen must persist or be repeatedly administered for acquired tolerance to be maintained.
4. Giving the antigen together with an immunosuppressant, such as cyclosporine, favours the development of tolerance.
5. Induction of tolerance is easier in the prenatal or neonatal period because of immaturity of the immune system.

N.B.: T cell tolerance is more easily induced and lasts longer than B-cell tolerance. → low doses → high doses

AUTOIMMUNITY

It is an adaptive immune response to self-antigens. Normally, this is prevented by autotolerance. Breakdown in autotolerance leads to production of autoantibodies and/or self-reactive T cells which may cause autoimmune diseases.

Aetiology of Autoimmune Diseases

Undoubtedly, autoimmune diseases have a multifactorial aetiology. There are a number of ways in which the self-tolerance mechanisms could be overcome:

- ① Exposure of the immune system to antigens that are normally sequestered within organs, e.g. eye lens and sperms. Lymphocytes specific to such antigens were not exposed to them during development and, therefore, were not deleted. For example, testicular trauma or infection may release sperm antigens which trigger the immune system. Antibodies formed will react with testicular tissue and inhibit spermatogenesis.
Sequestered antigen
- ② Structural modification or alteration of tissue proteins by drugs, chemicals or viruses, so that such antigens are no longer recognized as self; e.g. α-methyl dopa is thought to modify proteins on the surface of red cells leading to autoimmune haemolytic anaemia.
α methyl dopa drugs
- ③ Cross reactivity: Some strains of Streptococcus pyogenes share antigenic determinants with the heart tissue. Antibodies produced against such strains may react with heart tissue leading to rheumatic fever.
streptococcus share antigens with heart
- ④ Breakdown in the immune network which may occur as a result of:
 - Interference with the mechanisms which normally suppress surviving self-reactive T cells.
 - Polyclonal activation of lymphocytes: Certain agents (e.g. viruses or bacteria) are capable of non-specifically stimulating many clones of lymphocytes, including self-reactive clones.
 - Over production of IL-2 by Th1 cells.

Genetic predisposition to autoimmune diseases

Genetic factors appear to play a role in the development of autoimmune diseases, and autoimmune diseases are found to run in families. This is thought to be related to specific HLA (MHC) antigens which are important in presentation of antigens to T cells. There are strong associations between several autoimmune diseases and particular HLA specificities, e.g. systemic lupus erythematosus (SLE) with DR3, rheumatoid arthritis with DR4 and ankylosing spondylitis with B27.

Michele Knight



Spectrum of Autoimmune Diseases

There is a wide spectrum of autoimmune disorders. At one extreme, there are the organ-specific diseases where the immune response is directed against components specific to the organ involved. An example of this is autoimmune thyroid diseases: Grave's disease, Hashimoto's disease and myxoedema.

At the other end of the autoimmune spectrum are the non-organ-specific diseases where the lesion is not confined to one organ. Examples of such diseases are SLE and rheumatoid arthritis.

In between, antibodies formed are non-organ specific but the lesion tends to localize to a single organ e.g. antimitochondrial antibody in primary biliary cirrhosis.

A number of patients with autoimmune diseases tend to suffer from more than one condition.

Mechanisms of Tissue Damage in Autoimmune Disorders

1. Cytotoxic reactions (type II) as in cases of autoimmune haemolytic anaemia.
2. Immune complex deposition (type III) as in SLE and rheumatoid arthritis.
3. Cell-mediated reactions (type IV) as in cases of ulcerative colitis.
4. In cases of Grave's disease, anti-thyroid antibodies react with thyroid gland cells. This stimulates the secretion of excess thyroxine, leading to thyrotoxicosis.

Laboratory diagnosis of Autoimmune Diseases

1. Elevated level of serum immunoglobulins.
2. Detection of autoantibodies in the serum, e.g. rheumatoid factor (IgM directed against self-IgG), antinuclear antibodies (ANA), anti-DNA antibodies, anti-mitochondrial antibodies, anti-smooth muscle antibodies and anti-thyroid antibodies.
3. Detection of immune complexes in the serum and in tissue biopsy.
4. Decreased level of serum complement, due to uptake in immune complexes.

Management of Autoimmune Diseases

1. Anti-inflammatory drugs, e.g. corticosteroids.
2. Immunosuppressive drugs, e.g. methotrexate.
3. Plasmapheresis: clearing the plasma from immune complexes and antibodies.
4. Interference with the cytokine network is under trial.

Chapter 23

IMMUNODEFICIENCY DISEASES

In view of the complex nature of the immune response, it is not surprising that a wide array of immunodeficiency diseases exist. The individual may be born with an immune defect (**primary immunodeficiency**). More commonly, he may acquire a transient or permanent immunologic impairment later in life (**secondary immunodeficiency**).

A- Primary Immunodeficiency

I. Defects of the Innate Immune System

1. Phagocytic cell defects:

These may involve any of the stages of phagocytic function.

a- Defects of migration (mobility): An example of this condition is **leucocyte adhesion deficiency**, which involves a defect in **adhesion molecules**. This leads to failure of neutrophils and monocytes to migrate from the blood stream to sites of infection, despite their presence in large numbers in the blood. Patients present with infections of the skin, mouth and respiratory tract, but with little pus formation (because pus is largely formed of neutrophils).

b- Defects of engulfment: These are usually associated with defects of mobility and are rarely found alone.

c- Defects of intracellular killing: **Chronic granulomatous disease (CGD)** is one of the most important examples. In this condition, phagocytes cannot produce reactive oxygen radicals, leading to decreased ability to kill intracellular as well as ingested extracellular bacteria. It is usually inherited as an X-linked disease and affected male children present with chronic bacterial infections which in some cases may lead to the formation of granulomas.

2. Complement deficiencies

a- Defects of the early components of the classical pathway of complement activation:

These components are important for the elimination of immune complexes. Thus, their deficiency leads to accumulation of immune complexes, and local tissue damage.

b- Defects of the early components of the alternative pathway of complement activation:

These are associated with pyogenic infections, since the alternative pathway is important for early clearance of these infections.

c- Deficiency of the terminal components of complement (C5-C9) (Membrane Attack Complex):

Effects of this deficiency are confined to increased susceptibility to the capsulated organism *Neisseria meningitidis*. This shows that for most extracellular bacteria opsonization by the early complement components is sufficient, and that lysis by complement is mainly important for bacteria such as *Neisseria*, which are capable of intracellular survival.

* Give example of phagocytic defect & explain effect
e.g. Neutrophils & macrophages
for phagocytosis & infection

* Mention Complement Component responsible for each of the following
 - Hereditary Angioedema $\uparrow$ - Immune complex clearance $C3b$
 or $\downarrow C1$ inhibitor $\downarrow$ - opsonisation $C3b$
 $C2a$ - MAC $C5b-9$
 d- Deficiency of complement regulatory proteins: - inflammatory reaction $C3a$ $C5a$

Absence of complement regulation can cause many problems. Deficiency of $C1$ inhibitor, for example, leads to uncontrolled activation of the classical pathway of complement activation, starting with $C1$, followed by $C2$ activation and generation of $C2a$ (a vasoactive amine). This causes fluid accumulation in the tissues, and swelling of the epiglottis, which may lead to suffocation and death. The condition is known as hereditary angioedema.

3. Deficiency of NK cells

This is usually present in conjunction with other defects and rarely alone. Since NK cells are important in anti-viral and anti-tumour immunity, patients with NK cell defects suffer from certain viral diseases and malignancies.

II. Deficiency of Specific Immunity

1. B cell immunodeficiency (antibody immunodeficiency)

The primary antibody immunodeficiency disorders range from complete absence of all classes of antibodies to selective deficiency of a single class or subclass of antibody.

a- X-linked agammaglobulinaemia: is a severe form of antibody immunodeficiency with marked deficiency or complete absence of all classes of antibodies. Infants with this condition usually become symptomatic following natural loss of maternal antibodies at the age of 5-6 months. They suffer from severe chronic bacterial infections such as otitis, bronchitis and pneumonia. Extracellular bacteria are responsible for most of these infections. Patients have a normal T cell response to viral infections, but susceptibility to some viruses may be increased due to the absence of neutralizing antibodies.

b- Transient Hypogammaglobulinaemia of Infancy (THI): is another example. All infants develop physiological hypogammaglobulinaemia at 5 - 6 months of age when maternal IgG decreases and the infants start synthesizing their own IgG. Sometimes, the infant may fail to start IgG synthesis at this time, resulting in a prolonged period of THI. Such infants have recurrent infections and poor responses to the vaccines taken routinely at that age.

2. T cell immunodeficiency

Defective T cell immunity, without affection of humoral immunity is rare, because of the collaboration between T cells and B cells in the process of antibody formation. Thus, production of antibodies against T-dependent antigens in particular can be affected in cases of T cell immunodeficiency.

Di George syndrome is a severe form of T cell deficiency caused by congenital aplasia or hypoplasia of the thymus. The patients develop recurrent and chronic viral, bacterial, fungal and protozoal infections.

3. Combined T and B cell deficiency

This involves defective T and B cells. It may be complete or partial. The complete form is called severe combined immunodeficiency (SCID). Patients present during the first few months with failure to thrive, continuous diarrhoea, as well as viral and fungal infections.

B- Secondary Immunodeficiency

Several disorders are associated with secondary immunodeficiency:

1. Malnutrition is the leading cause of immunodeficiency in the world as a whole, and is of particular importance in poor and under-developed countries.
2. Human immunodeficiency virus (HIV) causes acquired immune deficiency syndrome (AIDS). The virus infects CD4 Th cells.
3. Other virus infections may also cause transient immunodeficiency, e.g. measles.
4. Severe bacterial infections, e.g. Tuberculosis.
5. Parasitic infestations, e.g. Shistosomiasis.
6. Malignancies, especially those affecting the immune system such as Hodgkin's disease and leukaemias.
7. Chronic debilitating diseases, e.g. diabetes and renal failure.
8. Others, e.g. treatment with x-ray, cytotoxic drugs, steroids and immunosuppressive drugs, and burns with severe loss of body fluids.

Immunodeficiency should be suspected in the following cases

- Increased frequency of infections.
- Failure to clear infections rapidly despite adequate therapy.
- Dissemination of local infections to distant sites.
- Occurrence of opportunistic infections.
- Failure to thrive in infants and children.
- Development of certain kinds of tumours.

It should be remembered that the type of infection provides an important clue to the type of immunodeficiency disease.

Investigations in a Suspected Case of Immunodeficiency

Initial investigations

- Full blood picture.
- Measurement of serum IgG, IgM, IgA and C₃ by radial immunodiffusion.

Detailed Investigations

If initial tests are normal and high level of suspicion still exists, or if the initial tests point to a certain direction, then more specialized tests can be done:

T lymphocytes

- Quantitation of cells by fluorescein-labelled monoclonal antibodies against cell markers, e.g. with antibodies against CD3 (to quantitate total T cells) and against CD4 and CD8 (to quantitate T cell sub-sets).
- Measuring the ability of T cells to proliferate in response to mitogens. (Mitogens are substances that can non-specifically stimulate proliferation of lymphocytes. Some stimulate T cells only, some B cells only, while others stimulate both).
- Delayed type hypersensitivity tests to test the functional activity of T cells. Tests are done using extracts from organisms to which all people are frequently exposed, e.g. candidin test.
- Assessment of cytokine production.



B lymphocytes

- Quantitation of cells using fluorescein-labelled monoclonal antibodies against B cell markers.
- Determination of serum level of immunoglobulin classes as well as IgA in saliva.
- Immunization with commonly used vaccines such as tetanus toxoid followed by measuring the antibody response.

Complement

More detailed complement assays including total haemolytic assay of complement in serum. This involves measurement of the ability of test serum to cause lysis of RBCs coated with antibody, indicating the amount of functioning complement in the serum.

Phagocyte functions

Different tests can be done to assess phagocyte mobility, metabolic functions and ability to kill organisms.

Management of the Immunodeficient Patient

General measures

- Minimizing infections by avoidance.
- Administration of suitable immunizations. It should be noted that patients suspected of suffering from immunodeficiency should never be given vaccines containing living organisms, since this may be fatal.
- Prompt treatment of infections with antibiotics.

never give Immunodeficient living organism vaccine



Specific Measures according to defect

- Transplantation of foetal thymus or bone marrow in SCID and other severe deficiencies.
- Immunoglobulin therapy for cases of antibody deficiencies.
- Treatment with cytokines such as GM-CSF, IFN- γ and IL-2.

Severe Combined Immunodef.

Immunodeficiency disorders can be considered as "experiments of nature" which have helped scientists to understand the role of each component of the immune system against various infectious agents.

ANTIGEN-ANTIBODY INTERACTION

Serological reaction

In vitro antigen-antibody reactions (i.e. serologic reactions) provide methods for the diagnosis of diseases and for identification and quantitation of antigens and antibodies.

Characters of Antigen-Antibody Reactions

1. A reaction between antigen and antibody is specific, i.e. an antibody can combine only with the antigen which induced its formation. So, an unknown antigen (or antibody) can be identified by reacting with the known antibody (or antigen).
2. The same antibody reacting with the same antigen under different conditions may give different observable results. For example, antibodies against pneumococcal capsule reacting with capsulated pneumococcus would give rise to agglutination, while the same antibody reacting with purified capsule extract will lead to precipitation. Also, if RBCs are mixed with their antibodies, clumping of RBCs (agglutination) will occur. If complement is added to this reaction, lysis of the red cells will occur. So, the same antigen-antibody system can give agglutination in one condition and lysis in another condition. Thus precipitation, agglutination, lysis and other observable reactions reflect types of reactions rather than types of antibodies.

3. Serologic reactions can be performed not only qualitatively to determine whether antibodies are present or not, but also quantitatively to determine the amount of antibodies present. This is done by preparing different dilutions of the patient's serum in normal saline, e.g. 1/10, 1/20, 1/40 ... etc. and then mixing each dilution separately with a constant amount of known antigen suspension. The highest dilution (lowest concentration) of the patient's serum giving positive reaction is called the "**antibody titre**".

For the diagnosis of infectious diseases, two serum samples separated by 7-10 days interval should be tested. A **rising antibody titre** of four-fold (two dilutions) or more means that the antibody response is increasing with progress of illness. This indicates active infection.

4. Zone phenomenon: (Fig. 40)

Observable union between antigen and antibody occurs best when both reactants are present in optimal proportions. No visible reaction occurs in the presence of antigen excess or antibody excess.

If a series of tubes is set up, each containing a constant amount of antigen, and decreasing amounts of antibody are added to the tubes in the row, a reaction will start to appear in the tubes, gradually increasing along the row, reaching a maximum, and then falling off with the lower antibody concentration.

In the first few tubes of the dilution series, small soluble complexes are formed due to presence of antibody excess, while small soluble complexes are formed in the last few tubes of the dilution series due to presence of antigen excess. In between, visible large aggregates occur.

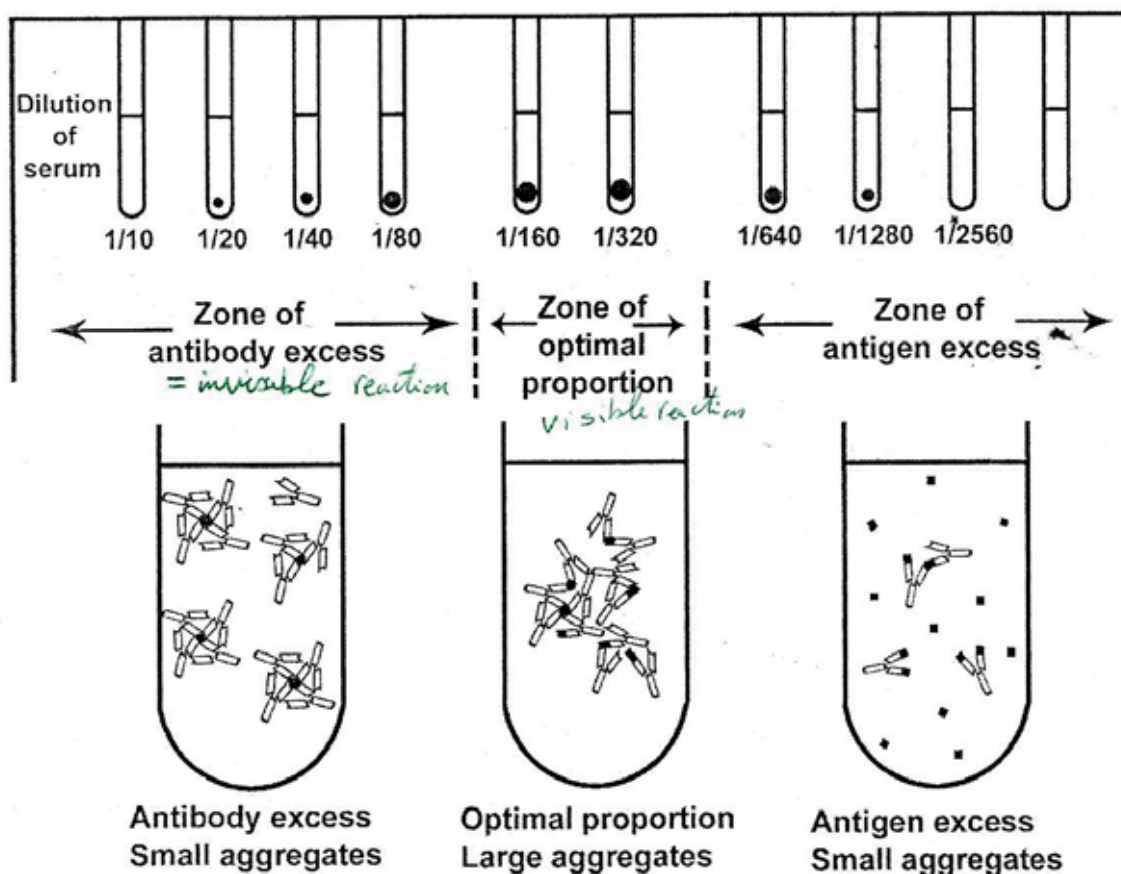


Fig. (40): Zone phenomenon.

Factors affect Antigen Antibody reaction

- The nature of the reaction between antigen and its antibody is mainly physical involving electrostatic forces. It generally requires the presence of electrolytes, e.g. NaCl, and a suitable pH around neutrality. It can occur anywhere between 4°C and 55°C, but certain antigen-antibody systems require certain temperatures for optimal reactions to take place.

Methods of Detection of Antigen-Antibody Reactions

I. Reactions Accompanied by Visible Phenomena

In these reactions, the resulting antigen-antibody complexes can be seen either by the naked eye or under the microscope.

Agglutination
Passive
Coombs test
Ragglutination inhibit.
precipitation
flocculation
Toxin neutralization
Complement fixation
Viral neutralization

Agglutination ←
 Brucella Agglutination + zone phenomenon

Define

A- Agglutination

A reaction between antibody and its antigen will result in agglutination (clumping) if the antigen is cellular or particulate (Fig. 41). Under these conditions, the antigen is called "agglutinogen" and the antibody "agglutinin".

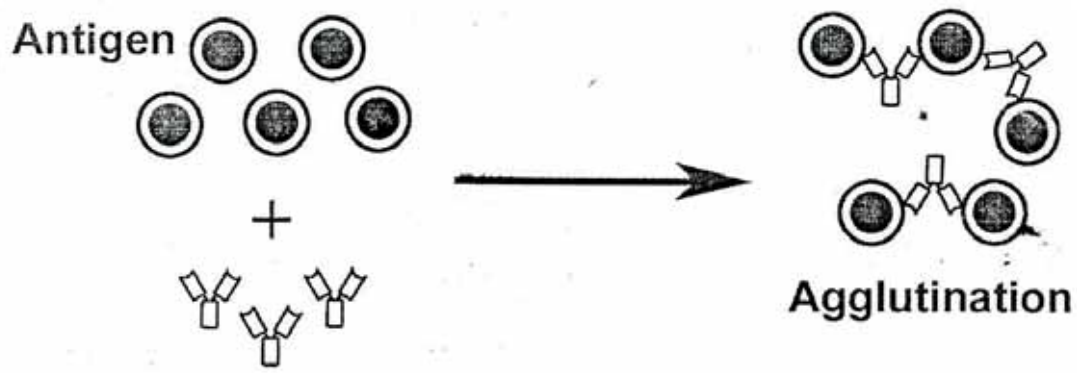


Fig. (41): Agglutination.

Agglutination tests have many clinical applications in medical practice. Generally speaking, since the reaction between agglutinogen and agglutinin is specific, therefore, if one of them is unknown we can identify it with the help of the other.

Agglutination reactions can be performed by 2 different ways:

1. Slide Agglutination (Qualitative Test)

This test is used to detect or verify the presence of an unknown antigen by means of a known antiserum. Thus, the exact antigenic type of a red cell or bacterium can be identified with standard specific antiserum. A drop of particle suspension (antigen) and a drop of antiserum (antibody) are mixed on the slide and the slide is gently rocked for few minutes; clumping will be observed in positive cases (if antiserum is specific for the antigen).

2. Tube Agglutination (Quantitative Test)

This test is commonly used in the serological diagnosis of infectious diseases, i.e. detection of antibodies against a certain pathogen; e.g. a patient suspected on clinical grounds to be suffering from typhoid fever would be expected to have high level of antibodies against *Salmonella Typhi* in his serum by the second week. His serum is taken (unknown antibody) and is mixed with a suspension of known typhoid bacillus. If agglutination occurs, this can be taken as indirect evidence for the diagnosis of typhoid fever. The test is usually performed quantitatively by using serial dilutions of the patient's serum to determine antibody titer.

Slide agglutination
 Tube agglutination



B- Passive Agglutination

It is the conversion of a reaction system that results in precipitation to one that results in agglutination, thus yielding a more sensitive indication of antibody or antigen.

This is done by adsorbing the known reactant (soluble antigen or antibody) passively on the surface of inert particles (e.g. latex particles or RBCs); thus either the antigen or the antibody will become particulate resulting in agglutination instead of precipitation (Fig. 42).

If RBCs are used as inert particles, the reaction is called passive haemagglutination.

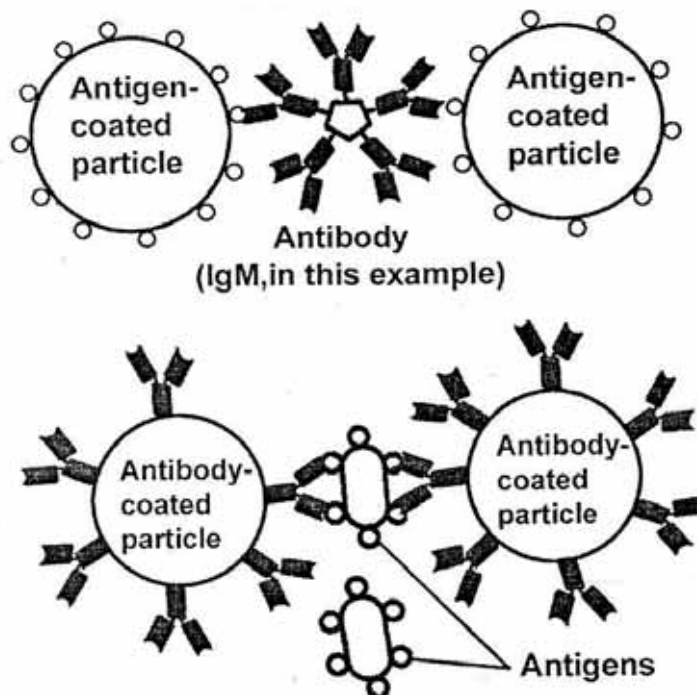


Fig. (42): Passive agglutination.

Examples of passive agglutination tests include:

1. Diagnosis of rheumatoid arthritis in which the patient produces an antibody (mainly IgM) to his own IgG. This antibody is called rheumatoid factor (RF). Agglutination of latex particles coated with IgG when mixed with patient's serum indicates the presence of RF (positive test).
2. Detection of C-reactive protein as an indicator of inflammation. Serum of patients agglutinate latex particles coated with anti C-reactive protein antibody.
3. Immunologic pregnancy test where latex particles are coated with anti-HCG (human chorionic gonadotrophic hormones). A drop of urine is added to a drop of coated latex. Agglutination occurs if HCG is present in urine (positive pregnancy test).

4. Diagnosis of **rheumatic fever** (a post-streptococcal disease) is done by detecting antibodies to streptolysin O toxin; these are formed in response to infection with *Strept. pyogenes*. Patient's serum is added to latex particles coated with streptolysin O toxin. Agglutination denotes presence of antistreptolysin O (ASO) antibodies. The test is done quantitatively by using serial dilutions of the patient's serum to determine ASO antibody titer (up to 200 units is considered normal).

C- Coomb's (Antiglobulin) Test

*for detecting RH & erythroblastosis foetalis

Sometimes antibodies to red cell antigens react with the cell surface but can not bridge between two RBCs to give direct agglutination (non-agglutinating antibodies). This occurs commonly with anti-Rh antibodies and autoimmune haemolytic anaemia.

It is, however, possible to show that the red cells are coated with antibody by adding an anti-human globulin (antibody against human immunoglobulin) which will bring about agglutination of the cells. This is the basis of Coomb's test which is performed in two ways (Fig. 43):

1. Direct Coomb's Test

AB درون بی → دانه و رسوب

This test is performed to detect non-agglutinating antibodies coating the red cells of newborns with erythroblastosis foetalis or patients with autoimmune haemolytic anaemia. The patient's RBCs are first washed and the antihuman globulin is added to the cell suspension. Agglutination occurs in positive cases.

2. Indirect Coomb's Test

AB درون بی → دانه و رسوب

This test is done to detect circulating non-agglutinating antibodies in the serum of Rh negative mothers sensitized with Rh antigen. The serum sample is added to Rh-positive, group O red cells and incubated. After washing, antihuman globulin is added. Non-agglutinating antibodies (if present in the patient's serum) will bind to the red cell surface and the antiglobulin will link them together giving visible agglutination.

D- Haemagglutination Inhibition Test

Haemagglutination involves the agglutination of red blood cells by antibodies (i.e. haemagglutinins), certain virus particles (e.g. influenza and mumps viruses), or other substances.

The ability of certain viruses to cause haemagglutination can be used for diagnosis of such viral diseases, since specific anti-viral antibodies bind to the viral particles and block their ability to agglutinate RBCs.

For example, if a patient is suspected of having influenza, his serum is examined for specific antibodies. Serum is mixed with known influenza virus and red blood cells:

a- If antibody is present, haemagglutination will be inhibited.

Antiviral antibody + virus + red blood cells = No Haemagglutination.

patient

b- If no antibody is present, haemagglutination will occur:

Virus + red blood cells = Haemagglutination.

Not patient

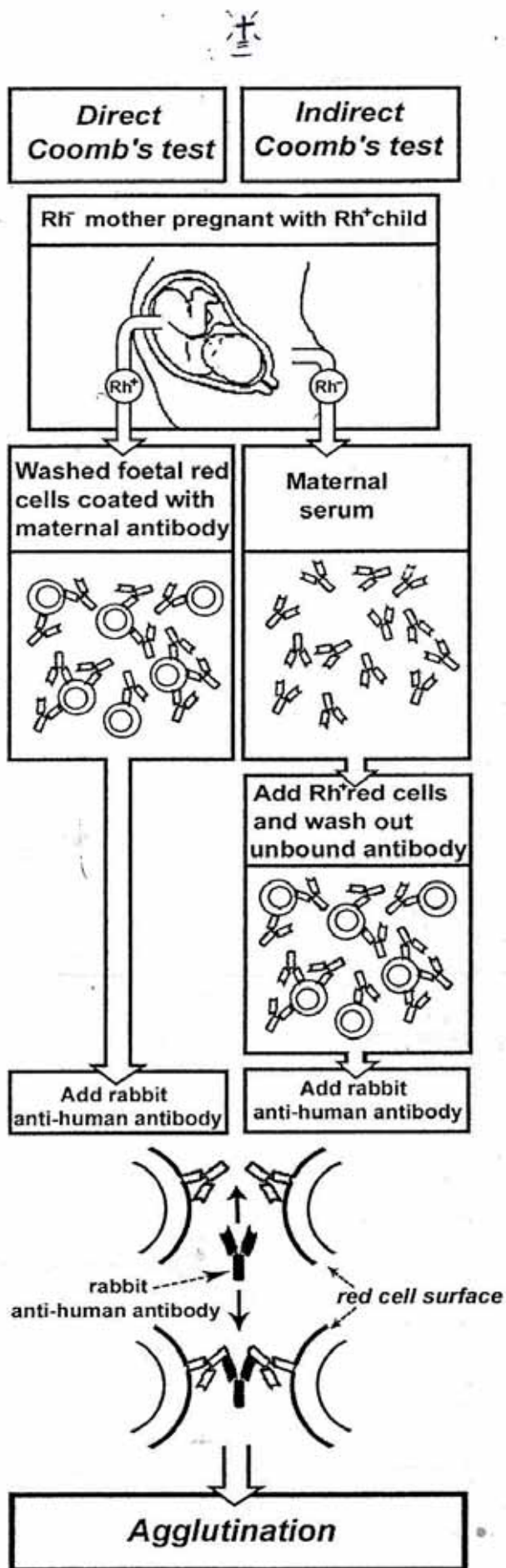


Fig. (43): Coomb's test.

E- Precipitation

Precipitation occurs when the antigen is soluble instead of cellular; therefore, a large number of molecules are required for lattice formation, and a large lattice must be formed for an aggregate to be visible.

Applications

1. Ring test

This test is done either in test tubes, in which the antigen is carefully layered over the antibody, or in capillary tubes, in which the antigen is pipetted in by capillarity followed by the antibody. A precipitation ring will form at the interface if the antigen and antibody are specific. This test can be used qualitatively for detecting and identifying antigens.

2. Agar gel diffusion

In this technique, antigen-antibody reaction takes place in semisolid media (e.g. agar). It can be performed in 2 different ways:

a- Double diffusion

1. Antigen and antibody preparations can be placed in separate wells that are cut in a thin layer of agar in a Petri-dish. The reactants diffuse towards each other through the agar. Where they meet at optimal proportions, bands of precipitate are formed.

2. Elek's test:

→ not neutralization test ←
This test is used for demonstrating the toxigenicity of a given strain of *C. diphtheriae* (Fig. 44). The strain tested is streaked on the surface of a serum agar plate. A strip of filter paper soaked with antitoxin is placed perpendicular to the organism. After incubation, the appearance of precipitation lines at the angles indicates toxigenicity of the strain (lines occur where the toxin and antitoxin meet at optimum proportions).

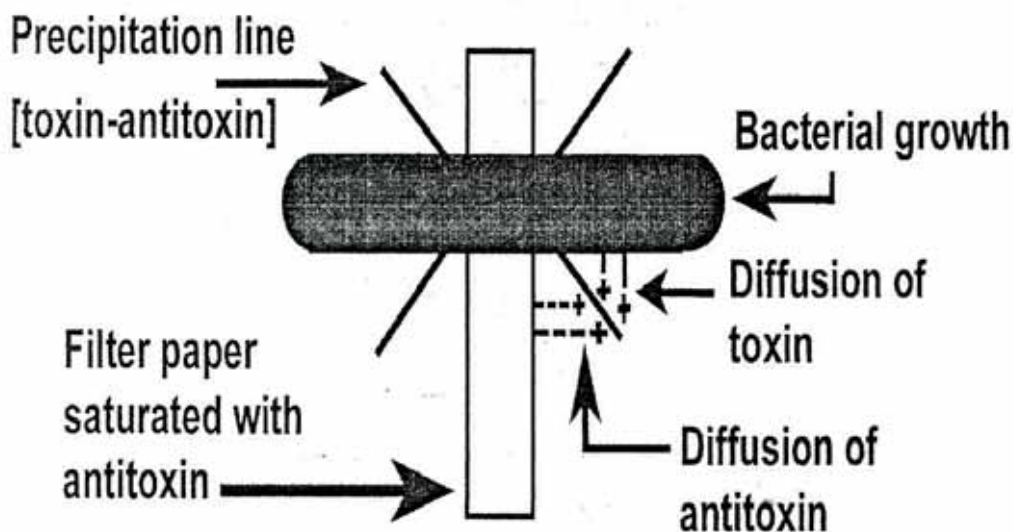


Fig. (44): Elek's test.

b- Single radial immunodiffusion

This is used to quantitate antigens. In this technique, the antibody is mixed with the agar (before pouring it in the plates), and the antigen is placed in wells punched in the agar gel. The antigen diffuses radially from the well until a point is reached where its concentration is optimal for precipitation to occur. Precipitation will show up as a ring around the antigen well. The diameter of the ring is proportional to the concentration of the antigen in the well (the greater the initial concentration of the antigen in the well, the further it must diffuse to reach the state of optimal proportions with the antibody in the gel) (Fig. 45).

This technique is routinely used to quantitate various immunoglobulin classes in human serum samples, e.g. quantitation of IgG (antigen of unknown concentration) by using agar gel mixed with anti-IgG antibody.

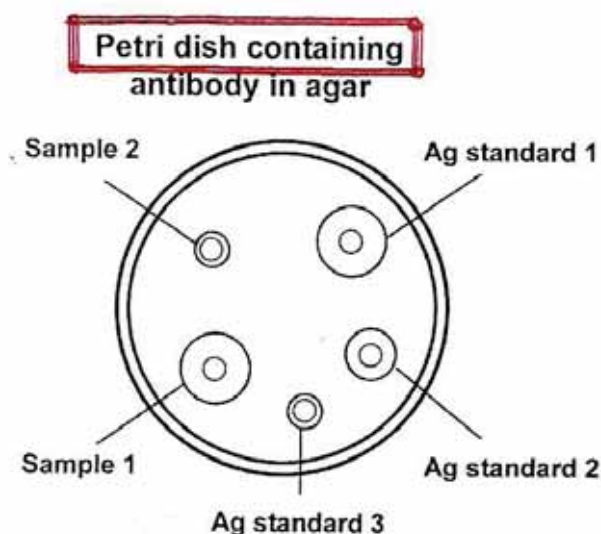


Fig. (45): Single radial immunodiffusion.

non treponemal test
in Syphilis ✓ = 5:3

F- Flocculation

antigen - antibody reaction
neither cellular nor soluble

This is another form of antigen-antibody reaction that occurs if the antigen is neither cellular nor soluble, but is a small insoluble particulate antigen.

The Venereal Disease Research Laboratory (VDRL) test is a slide flocculation test used for the serological diagnosis of syphilis. Instead of using treponemal antigen (which is difficult to obtain), the antigen used is water-insoluble cardiolipin. This forms microscopical aggregates in the presence of a heterophil antibody called reagin that forms in the serum of patients with syphilis.

In the rapid plasma reagin test (RPR), finely divided carbon particles are added (to enable the result to be read by naked eye).

tests uses antihuman globulin
② indirect immunofluorescence 121
② indirect ELISA

G- Toxin Neutralization

Antibodies against a bacterial toxin (antitoxin) can combine with the toxin and neutralize its deleterious effects.

An example of **in vivo** toxin neutralization test is that used to determine toxigenicity of *C. diphtheriae* (*in vivo* virulence test).

H- Complement Fixation Test

Complement is consumed (fixed) during the interaction of antigens and antibodies. This phenomenon forms the basis for the complement fixation test, which is a sensitive test that can be used to detect and quantitate antigens and antibodies.

Guinea pig serum, being rich in complement, is used in the laboratory as a source for complement.

Two systems are used in complement fixation test: (Fig. 46)

- a- Test system:** In the first step, the serum, which is heated to 56°C to inactivate native complement, is added to measured amounts of antigen and complement. If antibody specific for the known antigen is present in the serum, antigen-antibody complexes will form that consume (fix) all the complement. This initial reaction, however, cannot be seen.
- b- Indicator system:** In the second step, an indicator system consisting of sheep red blood cells (SRBCs) plus haemolysin (an antibody specific for SRBCs) is added, to test for the presence of free complement.

Interpretation of the test is based on the presence of haemolysis:

- If antibody is present in the patient's serum, all the complement will be fixed, and none will be free to lyse the SRBCs. This constitutes a **positive complement fixation test** (No Haemolysis = +ve test).
- If no antibody is present in the patient's serum, then the complement is not fixed and is free to interact in the indicator system and lyse the SRBCs. This constitutes a **negative complement fixation test** (Haemolysis = -ve test).

Complement fixation tests are used in the diagnosis of different bacterial (e.g. Wassermann reaction in syphilis), rickettsial, viral, fungal and parasitic diseases.

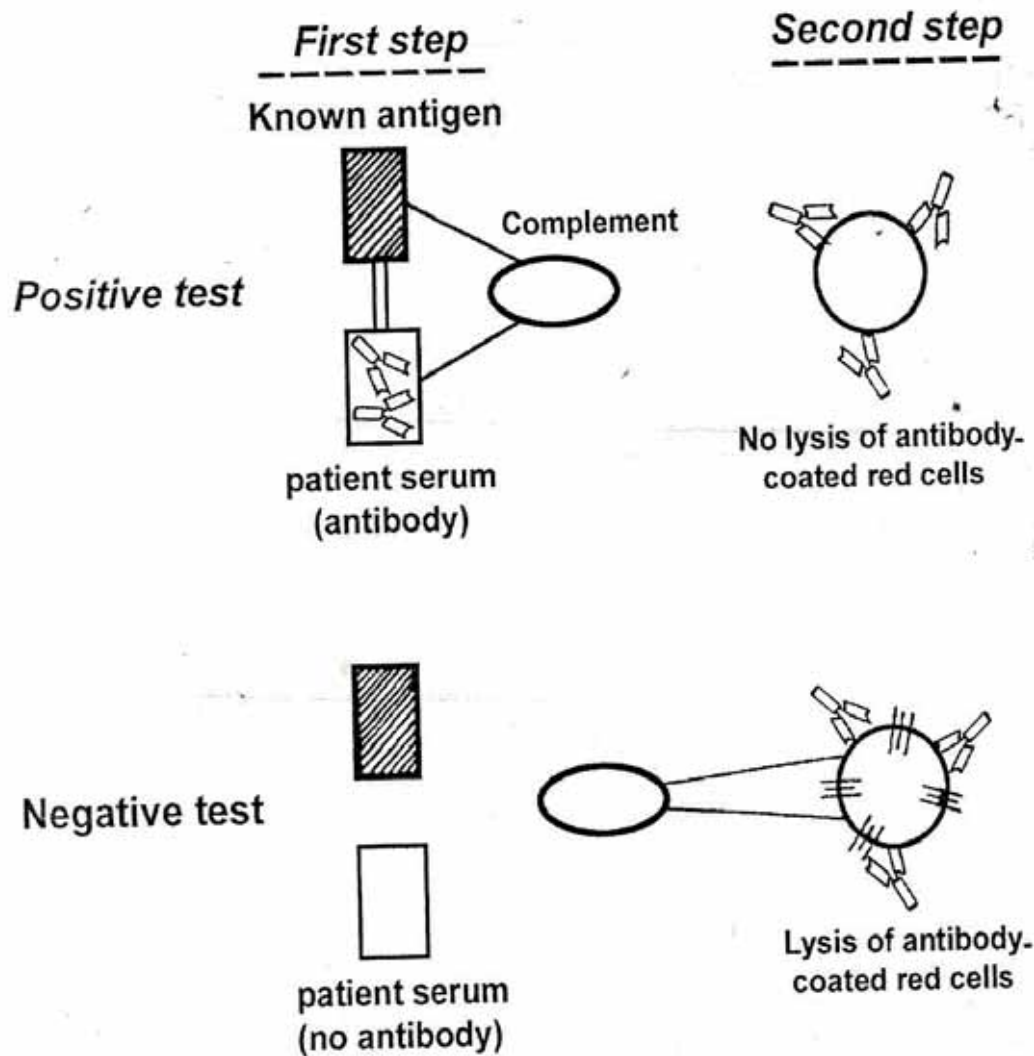


Fig. (46): Complement fixation test.

I- Viral Neutralization

Certain viruses, when added to appropriate cell culture, produce observable cell destruction referred to as **cytopathogenic effects (CPE)**. These effects can be inhibited by virus-neutralizing antibodies.

The phenomenon of CPE is useful in the search for virus-neutralizing antibodies in a serum sample. The serum suspected of containing antibody is added to a known virus suspension, and then a susceptible cell culture is inoculated with the mixture.

a- If no CPE develop, then antibodies were present in the serum sample.
Serum antibody + virus + target cell = no CPE.

b- If CPE develop, then no neutralizing antibodies were present:
Virus + target cell = CPE.

II. Reactions in which Antigen-Antibody Complexes are Detected by Labeled Reagents

A- Immunofluorescent Techniques

by UV microscopy
MCA

Fluorescent substances, e.g. fluorescein isothiocyanate, can be conjugated to antibody molecules to allow visualization of the molecules under ultraviolet light, using a fluorescence microscope. Such labelled antibodies may then be used to identify antigens. Both direct and indirect techniques are available (Fig. 47).

1. Direct Immunofluorescence: (Fig. 47a)

It is used to detect presence of a certain antigen in tissue sections fixed on a microscopic slide. Specific fluorescein-labelled antibody is used. If the antigen is present, the antibody will bind to it and will be visualized as a green stain on the specimen when it is examined under ultraviolet light. For example, in diagnosis of rabies in brain of rabid animals.

2. Indirect Immunofluorescence: (Fig. 47b)

This is usually employed to detect presence of antibodies against a certain organism in patient's serum using known antigen fixed on a microscopic slide. For example, in diagnosis of syphilis, the serum of patient (unknown antibody) is allowed to react with *Treponema pallidum* (known antigen) on a slide.

↳ of syphilis

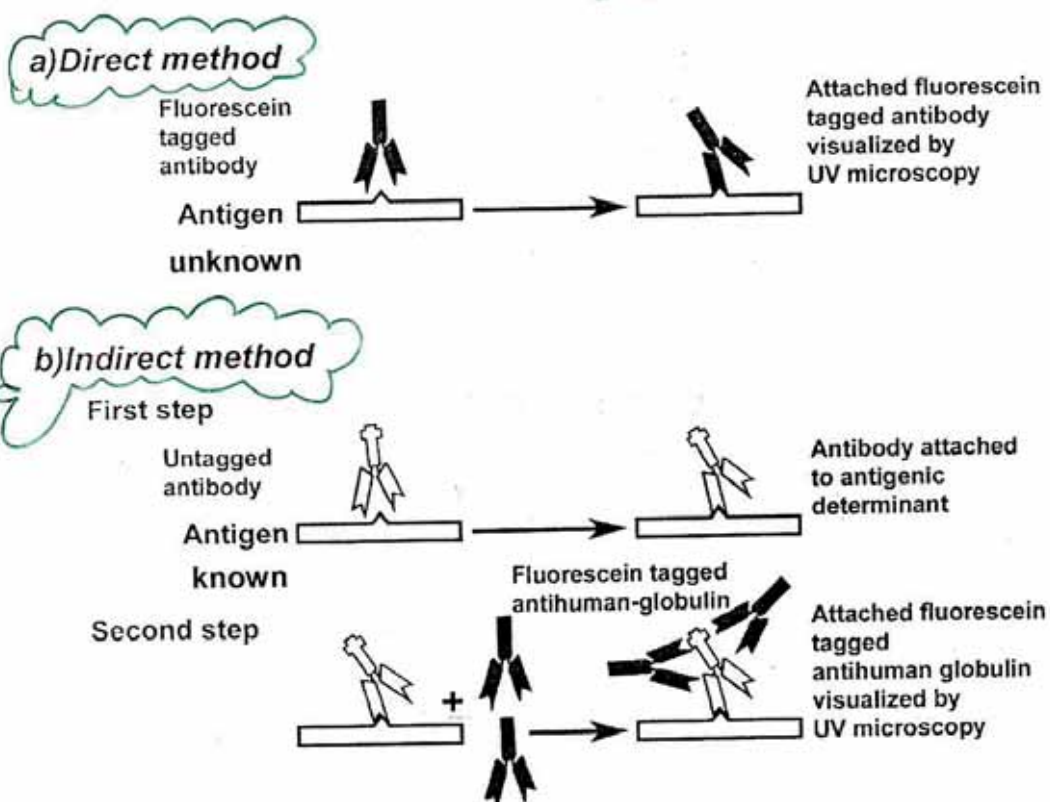


Fig. (47): Immunofluorescent technique.



After washing to remove unbound antibodies, the slide is overlaid with fluorescein-labelled antihuman globulin. This will bind to the antibody complexed to its antigen and will emit green fluorescence when examined by ultraviolet microscope.

B- Enzyme-Linked Immunosorbent Assay (ELISA)

direct
indirect

Principle

MCQ by colourimetry

ELISA is a highly sensitive and specific technique for the detection and quantitation of antigen and antibody. In ELISA, antigens or antibodies are conjugated to an enzyme (e.g. horseradish peroxidase, or alkaline phosphatase). The resulting conjugate is then both immunologically and enzymatically active. The presence of the antigen-enzyme or antibody-enzyme complex can be detected by addition of a proper colourless substrate, which in presence of the enzyme-becomes converted into a coloured product. The degree of colour change, can be measured spectrophotometrically, and is considered as an indicator for the amount of antigen or antibody in the sample.

There are a number of variations of the technique. The following are 2 examples:

- For the assay of an antigen, the double antibody technique (direct method) is used (Fig. 48a):

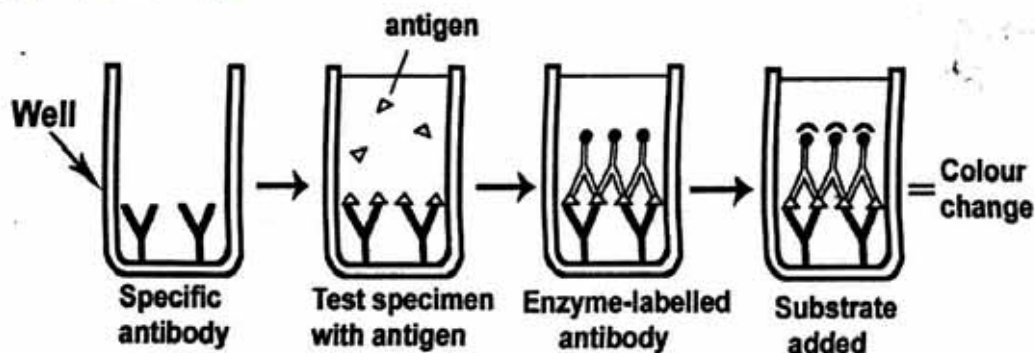
sandwich

- Known specific antibody is immobilized by adsorption onto a plastic surface.
- The clinical sample tested for presence of the antigen is added. If antigen is present, it will bind to the immobilized antibody.
- Any unbound material is removed by washing.
- An enzyme-labelled specific antibody is then added, which attaches to the fixed antigen.
- Excess unbound labelled-antibody is removed by washing.
- Finally, the enzyme substrate is added to detect the presence of enzyme-labelled antibody by the colour produced.

- For the detection of antibody (in serum sample), indirect method is used (Fig. 48b):

- Known antigens are immobilized by adsorption onto a plastic surface.
- The test serum is added. If specific antibodies are present, they will bind to the antigens.
- Non-specific unbound antibodies are removed by washing.
- An enzyme-labelled anti-human gamma globulin (conjugate) is added, which attaches to the Fc portion of the specific bound antibody.
- Excess unbound conjugate is removed by washing.
- The presence of bound conjugate can be detected colourimetrically by adding a substrate for the enzyme.

a) Direct method



b) Indirect method

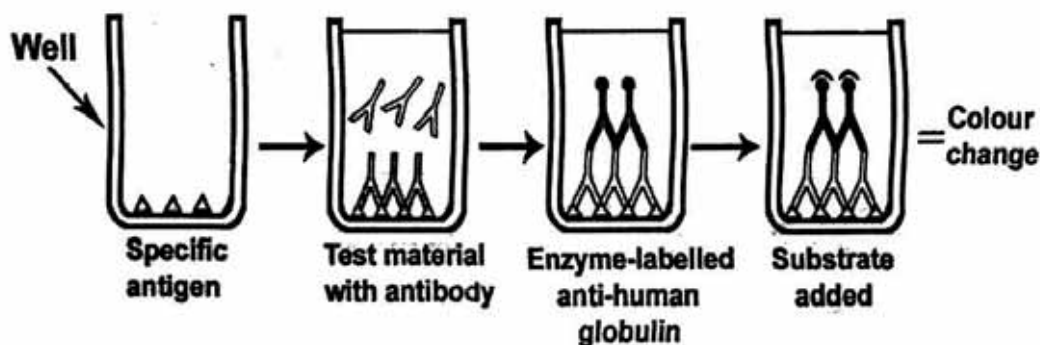


Fig. (48): Enzyme linked immunosorbent assay (ELISA).

C- Radioimmunoassay (RIA)

by radioactivity

In RIA, a radioactive isotope (e.g. iodine¹²⁵) replaces the enzyme in ELISA technique to label antigen or antibody. Antigen-antibody reaction is measured in terms of radioactivity.

RIA has the same sensitivity as ELISA, however, its use is accompanied by hazards of radiation. As in ELISA, there are several variations of the technique.

RIA is used to quantitate many biological substances, e.g. hormones, tumour markers, drugs . etc.

رقم الإيداع
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مها للطباعة والتجليد
إدارة/ محمد مبارك
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